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(*Recognosce notum, ignotum inspice*)

TRANSACTIONS

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B. BARNES and H. WORMALD

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MYCOLOGICAL CONTACTS

By R. W. MARSH, M.A.

This address will give some examples, taken mainly from personal experience, of the association of mycology with the studies included in agricultural research. The value of this association to the study of mycology and to the strength of this society is emphasized. A plea is made for corresponding associations with the medical and industrial fields touching mycology. Methods of promoting this end are briefly considered in relation to the general problem of the integration of the biological societies.

Mycology and agricultural science

It is possible for a government mycological service to stand detached from the general research and advisory service for agriculture. This happens, for example, in Canada, where a segregated Plant Pathological Service staffs a chain of laboratories in which not a solitary chemist or entomologist serves to leaven the mycological lump. But the usual British and United States system is one of association of mycologists with workers in other plant sciences in a research institute primarily established for an allied group of crops, as at Cheshunt, East Malling and Long Ashton. As the mycological service to agriculture expands, the question will undoubtedly arise whether this dispersal of mycologists shall continue or whether they should be grouped in an institute or institutes of plant pathology. There are obvious advantages in promoting the centralization of the legislative and regulatory branch of the service and of the fundamental investigations on fungicides. But in those aspects of mycology applied to agriculture that are now dealt with by the advisory and research mycologists, the proximity to other non-mycological workers gives valuable opportunities for co-operative investigations. Joint work is of great aid in arriving at balanced judgements.

The need for co-operation is apparent in plant-disease studies, where the field problems are invariably complex, their solution requiring assistance, on the biological side alone, from plant physiologists, chemists, soil scientists and entomologists. Where these workers are under the same roof, it is relatively easy for them to consult together and hammer out an agreed method of dealing with a plant pathological problem. This procedure is far more satisfactory to a grower than the alternative of leaving it to him to fit together the scattered bits of a solution of his problem as they arrive, first from one laboratory, then from another. In a plant-disease investigation it will often fall to the lot of the mycologist to assemble and present the unified conclusions of a group of workers. This is a very wholesome corrective of any one-sided approach to a question: an effective antidote to over-specialization.

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The study of the responses of plants to nutritional deficiencies is a rapidly extending zone in the environs of plant pathology but is not a domain to be termed mycological in the stricter sense. But the interaction of these nutritional factors with the expression of plant diseases caused by fungi opens up a wide field for joint investigations in plant physiology, pedology and mycology.

To quote from Dr Pethybridge's presidential address at Hereford in 1926, 'Although an enormous amount of work has been done on soils and manuring from a chemical point of view, very little of it has had any special bearing on plant pathology. It has often struck me that there is a field of work here for the pathologist well worthy of closer attention and one which might yield important and far-reaching results.' In considering this problem we have first the relatively straightforward examples, such as Club Root, where the degree of infection is largely determined by soil factors which may be modified by manurial applications, irrespective of the result of these applications on the plant. A more complex situation is exemplified by Mr Garrett's pot-culture work on the interactions between nutritional deficiency and *Ophiobolus* infection of wheat. He found, that in the absence of the fungus, deficiences of nitrogen and of potassium did not result in significant depression of yield compared with that from the full nutrient (NPK) series. Deficiency of phosphate, however, in itself greatly reduced yield. In a parallel series of pot cultures exposed to soil infection with *Ophiobolus*, root infection was least in the nitrogen-deficient plots and greatest in the phosphate-deficient, while the percentage infection of stem bases was lowest in the nitrogen-deficient and in the fully fed series. Comparing the infected plants with the controls, yield was reduced in every series except that receiving full nutrient. The greatest loss of yield due to disease was in the phosphate-deficient series, but significant reductions were also shown in the nitrogen- and potash-deficient infected cultures.

One reason that may be adduced for the accentuation of *Ophiobolus* attack by phosphate deficiency is that phosphate, more than any other fertilizer, encourages rooting. Plants with their root systems crippled by the Take-all fungus are able to produce fresh roots in phosphate-rich soil but cannot repair a loss of roots in soil where phosphate is lacking.

A further step takes us from the root-attacking fungi to those infecting only the above-ground portions of plants. A number of water-culture investigations and field trials have demonstrated that susceptibility to Mildew and Yellow Rust in wheat and to Mildew in barley was increased by heavy nitrogenous manuring and decreased by potash. In Chocolate Spot of beans, potash deficiency again is associated with increased liability to infection, and a team of workers at Wye has recently shown also that a highly significant relation exists between the resistance to attack in the field and the amount of available phosphate determined in the soil. A warning, however, against generalizing that phosphate deficiency and *Botrytis* attack always go hand-in-hand is afforded by the fact that *Botrytis* did not infect phosphate-deficient lettuce in pot culture experiments at Long Ashton but was most damaging in the calcium-deficient cultures.

Such results are not necessarily explicable on the assumption that a nutritional deficiency results in a weakly growing plant readily susceptible to disease. Apple Scab attack is reduced on the weakly growing trees kept short of nitrogen, although under orchard conditions there is the obvious complication that the tree making restricted growth has a less dense head of foliage than the fully fed tree and so dries more rapidly after rain. This, however, is not the sole factor concerned, as Muskett, Horne and Colhoun, working with Bramley's Seedling apples from Northern Ireland, have shown that nitrogenous manuring, with or without potash and phosphate, led to a marked increase in the nitrogen content of the fruit. This was directly correlated with susceptibility to scab on the tree and to rotting in store. The rate of radial advance of the apple-rotting fungus *Cytosporina ludibunda* was fifteen times greater on apples from the plot treated with nitrogen and potash than on those from the plot treated with potash alone.

Concerning infections of the leaves, it has been shown by Dr Katharine Johnstone that individual trees in pot-culture experiments demonstrate clear-cut relationships between nutritional status and scab susceptibility. Trees deficient in nitrogen show fewer infections and a much diminished persistence of individual lesions by comparison with those fully fed: those deficient in potash, on the other hand, exhibit enhanced susceptibility and increased persistence of lesions. Miss Johnstone's experiments on the toxicity of expressed leaf liquids, following earlier work by Dr Wiltshire, further indicated that the liquid expressed from apple leaves in the nitrogen-deficient series was more toxic to scab spores than that from the leaves of the trees receiving the remaining manurial treatments.

From results reported by Prof. Wallace on *Pseudopeziza* infection of black currants in relation to manurial treatments, a different picture emerges. Here, potash deficiency increases resistance to the disease while lack of nitrogen or of phosphate decreases it. This differentiation of effect on the Leaf Spot attack was of such magnitude that the anticipated adverse effect of omitting potash was partially outweighed by the unexpected beneficial action of this treatment in reducing the disease.

In controlling plant diseases, any practice whereby spraying might be reduced or eliminated is always worth attention, and this lends special interest to some of the interactions of manurial treatments and fungus infections. In Chocolate Spot of beans it is seen that resistance may be increased by correcting phosphate and potash deficiencies but that the occurrence of infection cannot be obviated by such treatment.

In other examples it is possible to attain something nearly approaching immunity but only at the expense of the general health of the host. In apples, Scab resistance is increased by lowering the nitrogen content of the leaves and fruit, and to a certain extent the benefits of spray applications can be supplemented by manurial treatments. But any attempt to reduce the nitrogen level to a point where spraying could be dispensed with would result in the production of a starved, stunted tree with fruits that were indeed uninfected but meagre morsels whose brilliance of complexion fails to compensate for a lamentable lack of flesh.

Again I have instanced black currant Leaf Spot where the attack can largely be averted by subjecting the bush to potash starvation. This too is dangerously close to the practice of curing the disease by killing the patient.

There are a number of records of the application, for the control of specific fungus diseases, of materials taken up by the plant, whose role in nutrition is still undetermined. G. T. Spinks showed in 1913 that the addition of lithium salts to a water-culture medium was remarkably effective in preventing Mildew on wheat grown in pot cultures. Dr N. L. Kent corroborated these observations in 1941 and demonstrated that watering with lithium chloride or nitrate at suitable concentrations reduced the amount of Leaf Spot disease in celery and at the same time increased the weight of the plants. This treatment also reduced the susceptibility of wheat seedlings to Brown Rust.

An analogous method of investigation is by the injection technique perfected at East Malling by Dr Roach. He has published an interesting example of the use of sodium thiosulphate for direct shoot tip injection of apple trees, with a resulting increase in resistance to Apple Mildew.

Such examples point the way to disease control by nutritional means, but at present it appears that nutritional treatments are likely to be useful adjuncts to other control measures, rather than substitutes.

A neighbouring arena into which one ventures with trepidation is that of the relation between fungus diseases and the source of manure, i.e. whether organic or inorganic. To hear some of the more fervent reciters of the compostolic creed, one would imagine that the research stations harboured a subversive organization of soil saboteurs, poisoners of the good earth, fosterers of pests and diseases, destroyers of fertility in plant, man and beast, whose final aim was a sterile world, thickly encrusted with sulphate of ammonia. But in the realms of fact there are no compost haters. The crux of the problem is, that supplies of organic manures are insufficient and have to be eked out. Even in a garden, working on a spade and wheelbarrow scale, it is rare to find that sufficient compost can be made and distributed annually to maintain a high level of soil fertility. And if we consider the problem of a farm with, say, fifty acres of arable land, the collecting, making, hauling and spreading of the tonnage of compost required for this acreage would require an expenditure of time, labour, water and transport far beyond the capacity of the equipment available. The farmer has every encouragement from the soil scientist to use organic manures up to the economic limit, but there is almost inevitably a deficit to be met between the nutrients removed by a crop and those returned in organic manures. These deficits can be economically made up only by the use of mineral fertilizers, and it is the work of the soil chemist to determine how this may best be done. It seems particularly hard that in these times the soil chemist should be accused of promoting an excessive use of 'artificials', when he has in the past five years worked in an environment of rationing and limitation of supplies and, more often than not, has been concerned to find out not how much, but how little inorganic manure, it was practicable to use.

All this may possibly be termed a diversion, but there is one point on which the mycologist feels he must take issue with those whom Prof. Salisbury has termed 'muck-minded'. Some of them have been known to state (for example) that potatoes grown with (and, by inference, because of) compost remain free from Blight, whereas those grown with inorganic manures succumb to the disease. Such statements might not seem worth controverting, but they are made so persistently and have apparently gained credence in such exalted quarters that there seems nothing for it but to carry out a series of *ad hoc* experiments to show where the truth in these matters is to be found.

An experiment recently described by Dr Croxall is of interest in this connexion. He grew onions in a series of plots given a selection of manurial treatments including farmyard manure, sludge with town refuse compost, a complete inorganic dressing, compost plus inorganic nitrogen, etc. One would expect that an onion bulb with its prolonged and intimate contact with the surface layers of the soil would have an excellent chance of contracting any fungus infection supposedly promoted by the use of inorganic manures. However, when Dr Croxall recorded the fungal rots in the stored onions he found that the percentages of rots from the onions grown with the treatments mentioned showed no significant difference due to manuring.

The study of the materials used for the control or prevention of fungus growth is one in which little progress can be made without the closest co-operation between the mycologist and the chemist. At first sight it might appear that such studies added little to our knowledge of the biological processes of the fungi concerned. However, these investigations have provided us with a completely new chapter of mycological knowledge concerning the exudates produced on the germination of fungus spores. Outstanding contributions to this elucidation of the mechanism of interaction between spore and fungicide have come from the partnership of McCallan and Wilcoxon at the Boyce Thompson Institute. The conception that spore exudates exist and play a major part in bringing copper into solution has greatly widened the choice of water-insoluble copper materials which may thus be made available as fungicides. A systematic study of this field by Dr Hubert Martin and his co-workers has led to the development of a wide series of commercial copper sprays whose suitability for amateur use has been of particular value in war-time. It is not too much to say that the intensive work on new fungicides in the past decade is revolutionizing the realm of spray materials.

I have touched only on the copper sprays and to go further in this direction would take me too far from my course. Where, however, the crop to be sprayed is of special value as a source of vitamin C (the fruit of black currants for example), the presence of copper spray residues antagonistic to this vitamin would obviously be undesirable. Hence the present interest in the development of non-metallic sprays such as the thiocarbamates, the aim being the derivation of a spray which combines satisfactory field performance with the absence of any residue adversely affecting the nutritional and processing value of the crop.

There are obvious advantages to be gained by the association of a mycologist with an entomologist, for example in the planning of spraying programmes. In reading accounts of tests of fungicides in American orchards it is useful to be reminded of the overriding claims of codling moth control in that country. Papers have been written from which the unwary reader would assume that the fungicides used were diluted with water in the accepted fashion, but on further inquiry it is discovered that the fungicides were in fact added to a suspension of lead arsenate, the use of arsenicals being such a matter of course in the American programme that the writer of the paper thought it not worth mentioning. More particularly, the results of American tests with various sulphur suspensions are subject to the reservation that these relatively weak fungicides are added to each of a long succession of arsenical applications which reinforce both the tenacity and the fungicidal effect of the sulphur deposits.

In this country, one of the weightiest reasons for the popularity of lime sulphur in the apple-spraying programme is the fact that this spray has a certain value in the control of Red Spider. The natural reluctance of the grower to apply two sprays when one will do has again led to the development of combined insecticide-fungicide applications and incidentally stimulated the study of wetting agents which were not, like soap, incompatible with lime sulphur. The success of the orchard entomologists in shifting much of their insect control programme to the relatively unhurried days of winter has led to investigations into the possibility of dealing also with apple scab at that season. Wide use is being made of di-nitro-cresol in winter washing and it has been shown by Keitt in Wisconsin that di-nitro-cresol spraying of orchard soil on which overwintered leaves are lying (the so-called 'floor spraying') prevents the maturation of apple scab ascospores in the treated leaves and thus renders easier the control of scab in that orchard in the ensuing season. While the problem is complicated in this country by the existence of unassailable shoot and bud infections on certain apple varieties, there are many plantations, for example those of Bramley's Seedling, in which scab infection in spring can arise only from overwintered leaves. Co-operation with the entomologist in trials employing di-nitro-cresol in such plantations might well give valuable results.

Other types of dual-purpose sprays are beginning to come into use. Manganese deficiency in apples is controllable by the addition of manganese sulphate to the routine lime sulphur applications. Materials for preventing pre-harvest drop of fruits (e.g. α-naphthalene acetic acid) might similarly be combined with late fungicidal applications designed to check storage rots.

The results of the association of mycologists with workers in other fields of agricultural science are not limited to securing a fuller understanding and a more rapid solution of the problems dealt with by joint study. The stimulus for the initiation of many classic mycological studies has come from the importance to agriculture of disease-producing fungi. It is highly unlikely that mycology would be enriched by so much detailed knowledge of *Puccinia graminis*, *Phytophthora infestans*, *Venturia inaequalis* and *Botrytis*

cinerea were they not pathogens of valuable agricultural crops. Further, the mycologist, working, for example, with a plant physiologist, has his interest aroused in the physiological aspects of the fungus under study and learns to adopt for his purposes the techniques developed by his colleagues. All the resulting information gained trickles back into and swells the main stream of mycological knowledge. If it is in order, I should like to quote this sentence from the Society's unpublished report on systematic mycology: 'The pathologist carries out experiments on the biology and pathogenicity of his fungus, during the course of which a body of valuable and essential data on the effect of host and other experimental conditions is obtained, and this is of the utmost reciprocal value to the systematist for determining new standards in classification and nomenclature.'

The point I have attempted to make is that the association of mycology with agricultural science has enlarged the content of pure mycology. This fruitful association is paralleled by the links between mycology and forestry, a subject needing no further treatment here since it was admirably covered by Mr Cartwright's Presidential Address in 1937.

Industrial and medical mycology

These agricultural and silvicultural sections of our subject I might group under the title of rural mycology. There exists also an urban mycology: mycology in its industrial and medical aspects. Considering only the main headings, there are the fungi attacking man and animals; those causing food spoilage and deterioration of textiles and paints. There are fungi used as sources of protein for human consumption, such as *Torulopsis utilis*, soon to be in large-scale production in Jamaica. Then the yeasts, no longer used only by brewers and bakers (I may add cider makers), but of fundamental importance in maintaining the production of industrial alcohol which is destined to play a more and more important part as increasing inroads are made on the world's petroleum supplies.

The large-scale culture of fungi for the utilization of their metabolic products is one of the aspects of industrial mycology in which developments have been most spectacular. Glycerol, acetone, citric and gluconic acids, ergosterol, invertase, quinones and mould pigments are but random examples of the products of the investigations (notably those of Dr Raistrick and his collaborators) in the biochemistry of moulds. And finally, we have the range of therapeutic derivatives on one of which, penicillin, a new industry has been based. Among the list of recipients of the Society's recent Report on systematic mycology were the Research Associations of the cotton, woollen, flour milling, printing, cocoa and chocolate, distilling and brewing industries, the Food Manufacturers' Federation, the Building Research Station and the Pharmaceutical Society. This list suggests a few of the directions available for the expansion of our interests.

In all these fields there are, as in rural mycology, workers who have accumulated knowledge of their fungi and have broadened this knowledge through contact with their colleagues in other sciences. It is reasonable to assume that urban mycology has much to contribute to our Society,

but I would suggest that we have room for a more widespread appreciation of the opportunities open in this field.

The development of contacts between academic mycology and its applied side is bound to be influenced by the place allotted to mycology in University curricula. The whole field of the methods of training of entomologists and plant pathologists is being actively studied by the Plant Pests Committee of the Association of Applied Biologists, whose report will be of great value in this connexion. A recent draft report on university research made by the Association of University Teachers also touches on this topic. This suggested the formation of Departments of Mycology, without, however, defining the status of the proposed departments. There is here, I feel, a possible danger to be guarded against in that the system existing in some American universities may creep in, whereby an undergraduate can specialize in mycology without having first laid any foundation of general biology. On the other hand, the present position of mycology in our under-staffed university departments of botany cannot be considered satisfactory. Some of us have been fortunate, but many students on looking back on their botanical training must be left with the impression that the fungi come in as a footnote to the phylogenetic morphology of the ferns. There is obvious room for mycologists who also know something of plant physiology, or of chemistry or of horticulture, but I know of few mycologists who have been able to find any use for the knowledge they have perforce accumulated of the Horsetails, the Mosses and the Seaweeds.

The links between botany and mycology in the British universities do, however, provide sufficient common ground for the mycologists to enter into the problems of plant physiologists, geneticists and other workers in agricultural sciences. There is, however, no system of training yet worked out suitable for the industrial mycologist, whose progress has been in directions where a botanical training provides little guide.

Individual members have already done much to establish links between our Society and these newer developments of mycology. There could hardly be a more comprehensive survey of the applications of fungi in industry and medicine than Dr Ramsbottom's address on the uses of fungi made to Section K of the British Association in 1936, supplemented by his 1944 lectures at the Royal Institution. Dr Wiltshire has just published a pioneer bibliography of medical mycology, and Mr George Smith's text-book of industrial mycology is indispensable. But, taking the Society's publications as a criterion, there are in the 3280 pages of the last ten volumes of our *Transactions* only forty-four which could be considered to treat of species selected for their industrial or medical interest.

The publication of work on the fungal therapeutants has now been for some years subjected to a war-time black-out. But it is at least to be regretted that none of the results of the pre-war investigations of the bacteriostatic action of fungi found their way into our *Transactions*. Many mycologists must find it humiliating to confess that their knowledge of penicillin has been gleaned almost entirely from the popular press. It is, of course, not a question of blaming individual workers for not beseeching the Society to hear them: the fault is in ourselves as a Society that our

scientific curiosity was not strong enough to overcome the difficulties of obtaining informed opinion on this section of our subject.

Now it would seem that chinks in the black-out are appearing, and some of the mycological details of penicillin production are being uncovered. A letter to *Nature* in August referred to penicillin-like antibiotics from a range of mould species. An article in the American Chemical Society's *Chemical and Engineering News* for 25 April of this year also records that penicillin is not exclusively a product of *P. notatum* but may also be formed by *P. chrysogenum*. Further, this article gives in reasonable detail the three most important production techniques and the methods for biological assay.

One unfortunate result of the apparent lack of interest of this Society in industrial and medical mycology is that the Society is not in any way considered when Government Committees are set up to deal with these matters—the Penicillin Committee for example. Again, when a Committee was first set up here to investigate fungal spoilage of military stores in the Western Pacific, this Society apparently remained as unknown to the Committee as the Committee did to this Society.

While much must still remain hidden, we can now begin to look back and discern outlines of the dramatic developments, the massive contribution, that mycology has made in the past five years. But while mycology has come to the fore, our Society, as a society, has not moved from the background. The war record of British mycology is exhilarating: that of the British Mycological Society is—well—blameless. Mycology has blazed up but we have not been illuminated.

It is not necessary to labour the point that it is in every way in the Society's interest to combat this attitude of apathy towards urban mycology. Widening our field of membership would confer obvious advantages on the Society. The Associate Member scheme is one valuable step in this direction: the other major source of recruits is in the rapidly expanding sphere of industrial mycology.

Relations with other societies

This, however, raises another problem which becomes more and more insistent yearly. There already exists a competition, not perhaps realized but none the less real, for the subscriptions of new entrants into the biological professions. With the probable post-war pullulation of scientific societies, this competition will become a matter of serious concern alike to old and to new biological associations. A young man or woman in his first post cannot be expected to contribute guineas to half a dozen scientific societies simultaneously. The resulting dissipation of effort has obvious disadvantages.

This is, of course, no new contention. It has been brought forward by many who are concerned over the future of our publishing societies, notably by Dr Martin in his recent presidential address to the Association of Applied Biologists. Again, as an example of the type of scheme which has been frequently suggested, I would call attention to Dr Darlington's

proposals. Dr Darlington envisaged a series of steps towards a system of federation, one of the first of these steps being an annual meeting of secretaries or delegates of biological societies to make arrangements for matters of joint interest in meetings, publications and general policy. In such a matter there is obviously the necessity for a lead to be given by an organization at a higher level than that of the individual societies. The name which comes first to one's mind is the Royal Society's National Committee on Biology. I do not know in what state of preservation this body is to be found, but as it has been functionless for some twenty-one years, there are grounds for assuming it to be now a vestigial structure. More, I trust, can be hoped of the newly proposed Biological Council sponsored by the Biochemical Society.

On such a central organization one might envisage some co-ordination of dates of meetings and of subjects between, for example, the A.A.B., the Microbiological Panel of the Society of Chemical Industry, the Society for Microbiology and our own Society. Members of any one of these societies should be welcomed at meetings of others in the group, and I hope that our Society, the senior of the four mentioned, would be the first in acting as host to the others. A further step, which might prove practicable in the near future, would be to extend our scheme of Associate Membership to include any full member of these societies without further qualification. This would make for larger and more varied audiences at our meetings, extending the links between systematic and applied mycology and making contacts valuable to our Society. There are people who have been using fungi simply as reagents, and some of these, whose concern hitherto has been only with what a fungus does, may be stimulated to find out what it is. As their interest develops they will wish to have our publications and to take up full membership.

Developments along these lines would help to overcome the immediate financial difficulties both of the societies and of their members, actual and potential. Further, as many new members would come in by introduction so to speak, from one society to another, it should do much to dispel any atmosphere of rivalry or disunity among those with interests in common.

I know that I have in many respects departed from the conventions of a Presidential Address, and I am conscious of having generalized on subjects of which I have no detailed knowledge. While the means are open to criticism, the end is plain—to recall attention to the first rule of our Society: that 'its object shall be the study of mycology in all its branches'.

STACHYBOTRYS AND *MEMNONIELLA*

By G. R. BISBY, *Imperial Mycological Institute, Kew*

The publication of my paper (1943) on *Stachybotrys* has been followed by the receipt of numerous cultures and collections. *S. atra* Corda proves to be common also in warmer areas and in the south temperate, and most of the cultures studied were like the 'standard' English culture I described in some detail. Singer's (1944) opinion, 'The theory of the prevalence of cosmopolitism in fungi ought to be abandoned altogether', may apply to boleti and agarics, of which he was writing, but does not apply to many moulds. It is true that there is variation in *S. atra*, but I have detected no evidence of 'geographic variation'.

Prof. G. W. Martin sent a specimen of *S. atra* from Iowa with the note that each of the phialides had a septum at the base. I could see quite clearly that he was right, and I doubtless should have been able to demonstrate septa previously, for I expected their presence. The phialides of *S. atra* seem to differ from the usual phialides only in their shape.

It happened that a paper by Hansford (1943), with three new species of *Stachybotrys*, appeared at about the same time as mine. I have now seen the type collections of his species. *S. kampalensis* Hansf., with conidia 10–14 × 6–8 μ, would formerly have seemed distinct from *S. atra*; but my interpretation of *S. atra* includes conidia of that size. *S. nephrospora* Hansf. is similar to *S. atra* except in having somewhat curved conidia. From the manner of formation of conidia of *S. atra* in heads, one might expect some curvature; but the fact remains that the conidia are usually symmetrical. Perhaps *S. nephrospora* will prove to be a distinct variety of *S. atra*. Cultures and further collections of these two species found in Uganda are awaited.

S. Theobromae Hansf. is an interesting discovery. The conidia are described as 20–28 × 15–18 μ on phialides 21–28 × 7–10 μ. My mounts showed that the phialides start to form in the manner usual with *Stachybotrys*, i.e. a terminal phialide is formed; another starts just below the septum which delimits the first, and is in turn provided with a septum; then a third phialide commonly develops. As Hansford states, no more than three phialides are evident. In the dried material mounted in water I did not see a spore longer than about 24 μ, and many are 17–20 × 15–17 μ. This collection is the second of a large-spored *Stachybotrys*, the first being *S. crassa* Marchal described fifty years ago in Belgium with 5–7 phialides 17–21 × 10–12·5 μ, spores globose, 16–18 μ. Marchal attempted cultures, but did not obtain conidia. It seems possible that, if this large-spored species shows variability comparable to that found in two other species of *Stachybotrys*, *S. crassa* and *S. Theobromae* may prove to be the same species; while we suspend judgement, we can tentatively add one species to the two I recognized previously.

I have seen only one more collection, found on germinating rice seed

in Uganda, of *S. subsimplex* Cooke. The description and figures of *S. Voglinii* Cif. (1922, p. 48) suggest *S. subsimplex*. I suspect that some of the recent reports of *S. papyrogena* (Sacc.) Sacc. refer to *S. subsimplex*, but I do not yet know what *S. papyrogena* is.

Dr Machacek, who supplied the 'pink *Stachybotrys*' I described briefly, writes that he has seen a few more similar cultures from soil in Manitoba. It may, therefore, prove to be a good new genus and species, but I have seen no more collections or cultures.

Cultures and data from Dr Lilian Fraser of Sydney and R. M. Brien of Auckland show that *Memnoniella echinata* (Riv.) Galloway is a common fungus, especially on old cloth in certain warmer areas. It regularly produces persistent chains of conidia without slime. The cultures agree with Galloway's description and culture, and the fungus is distinct from *Stachybotrys subsimplex*.

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A NOTE ON MEDLAR CLUSTER-CUP RUST (*GYMNOSPORANGIUM CONFUSUM* PLOWR.) IN KENT IN 1943 AND 1944

By M. H. MOORE, *East Malling Research Station*

(With Plate I)

Plowright's description (1889) of the aecidial stage of this rust was based upon infections artificially induced on *Crataegus oxyacantha*, *Mespilus germanica*, and *Pyrus* (*Cydonia*) *vulgaris*, with teleutospores from the perennial mycelium in *Juniperus Sabina*. Fischer (1904) also described it; he produced aecidia on the same three hosts and on *Crataegus monogyna* and, 'more seldom', on *Pyrus communis*, the teleutospore hosts being *Juniperus Sabina* and *J. virginiana*.

Its natural occurrence on medlar evidently is not common, for only two references to it can be found in the literature: (i) by Fischer (1904) in Berne in 1885 and (ii) by Wormald (1931), who reported that 'Medlar Cluster-Cup Rust (*Gymnosporangium confusum*) was present on a few leaves of a medlar tree on this Station, and on a single quince leaf', which thus appears to be the only record for Britain.

A severe attack was seen in July 1943, on the leaves and fruits of a medlar tree in an East Malling garden. The tree, a standard, was about 10 ft. high and 12–15 ft. in diameter, and near it to the east were two bushes, carpeting the ground, of a small-leaved variety of *Juniperus Sabina*. The nearer one was about 12 ft. from the medlar, the east side of which was much more severely affected than the west, and on this savin was found a shoot with a fusiform hypertrophy characteristic of infection on juniper. Though sterile when examined, it may have borne teleutosori earlier. It has been established that the medlar was from a batch of young nursery trees on one of which Wormald first observed the disease. They were at that time on a plot just outside this garden where the two savins were already growing. The rust has probably been present on this tree many times since, passing unnoticed until 1943.

Symptoms. The scattered leaf-spots, as seen on the upper surface, were roughly circular, slightly convex, and about 0·5 cm. in diameter. Most of them, brown in the centre, were surrounded by a conspicuous yellow, orange-streaked halo (Pl. I, fig. 1), and many bore spermagonia. The yellowish to pale brown, curved, cylindrical aecidia, up to 4 mm. long, arose from slightly thickened corresponding areas on the lower surface; they were occasionally found also on the upper surface. There were many affected fruits among the heavy crop and they bore similar aecidia on swollen areas, mostly on the fruit itself but sometimes on the calyx.

By October affected fruits had all dropped, leaving a good crop of healthy ones. There was no sign of leaf-drop in spite of the numerous

infections. The lesions had not developed since July and even severely affected leaves were turgid; the aecidia had dried up, leaving little trace. There was much healthy new foliage, and the disease had evidently not progressed after teleutospore production ceased.

In 1944 infection had already occurred by early June, but it was slight compared with that in 1943. Aecidia were present, but mostly not mature, and they were shorter (*circa* 2 mm.) than those of 1943. On incubation for a day or so in moist air they matured and split throughout their length, releasing clouds of spores. The savins were searched for teleutosori, and several shoots were found with fusiform hypertrophies about 5 cm. long. Parts of the bark had split and curled back revealing pale, cortical swellings up to 4×3 mm., but no teleutosori were seen on the dry field specimens. Incubation in moist air, however, induced a few pale, gelatinous spore masses to arise beneath the flaps of bark, and revived a few brown teleutosori about 5 mm. long (Pl. I, fig. 2).

The fungus. Spore measurements conformed in general to those of Plowright and of Fischer, but showed slight differences.

(i) *Thick-walled teleutospores*, $38–50 \times 20–26\,\mu$, av. $44 \times 24\,\mu$ (Plowright: $40–50 \times 20–25\,\mu$; Fischer: $35 \times 25\,\mu$), brown, broadly oval to fusiform, two-celled, with long pedicels.

(ii) *Thin-walled teleutospores*, $40–50 \times 19–24\,\mu$, av. $45 \times 20\,\mu$, with orange, granular contents.

(iii) *Basidiospores*, $4–6 \times 2–3\,\mu$, oval, hyaline.

(iv) *Aecidiospores*, $22–26\,\mu$, av. $24\,\mu$ (Plowright: $15–20\,\mu$; Fischer: $21–24\,\mu$), pale brown, globose, minutely verrucose.

Grove (1913) gives: (i) $30–50 \times 20–25\,\mu$, (iv) $21–24\,\mu$.

Plowright remarked of *Gymnosporangium confusum* that 'this species has hitherto been confounded with *G. Sabinae*, which it resembles in many points'. It has the same teleutospore host (*Juniperus Sabina*), but the sole aecidial host of *Gymnosporangium Sabinae* is stated by Fischer to be *Pyrus communis*, a 'difficult' host for *Gymnosporangium confusum*. Other differences (from Grove, 1913) are that in *G. Sabinae* the aecidiospores are larger ($28–30\,\mu$), the aecidia are flask-shaped, not cylindrical, the teleutosori are longer (8–10 mm.), and the teleutospores, while about the same length, are slightly broader ($40–50 \times 25–30\,\mu$) than those of *G. confusum*. According to Fischer, *G. confusum* is biologically closely akin to *G. clavariaeforme*, the aecidial hosts of which are *Crataegus oxyacantha*, *C. monogyna*, and *Pyrus communis*, with *Juniperus communis* as the teleutospore host. The aecidia of this species resemble those of *Gymnosporangium confusum*, but the aecidiospores, like those of *G. Sabinae*, are larger ($28–30\,\mu$).

Weather relations. The following data concerning the distribution of wind and rainfall in May, when medlar leaves have unfolded, are relevant to the severity of infection in 1943 and its comparative lightness in 1944. As the medlar was to the west of the savins, the basidiospores, after being forcibly abjected from the basidium (Coons, 1912; Buller, 1924), would need easterly winds in May to carry them up to the medlar. May 1943, with easterly winds on eleven days, and a total rainfall at East Malling of 2·82 in. (1·01 in. on 1st), was alternately dry and wet, with mostly dry

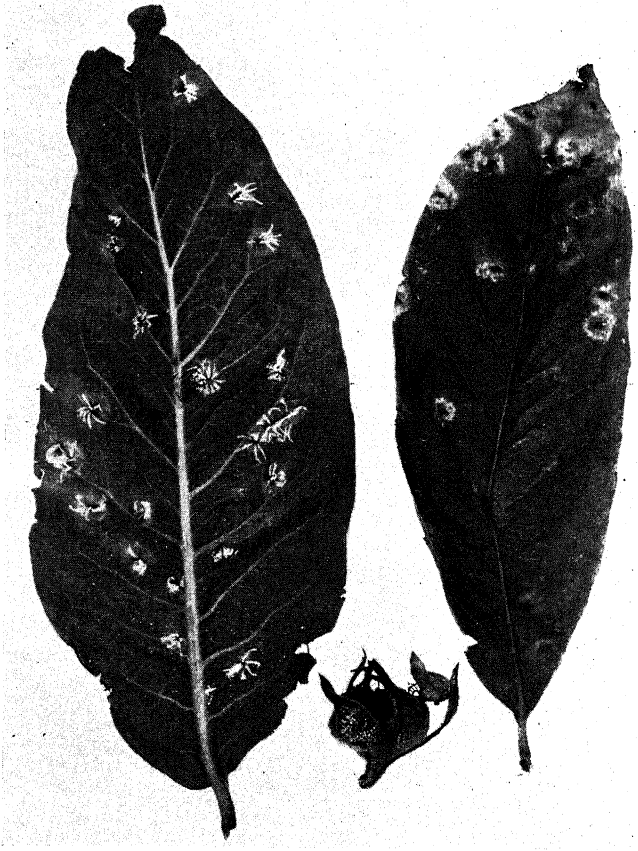

Fig. 1.

Fig. 2.

easterly winds during the first and third weeks, and mostly wet westerly ones during the second (1·29 in.) and fourth (0·47 in.). The basidiospores, produced under the stimulus of moist conditions, could thus have been continually carried up to the medlar shortly before a rainy spell during at least two periods. May 1944 had easterly winds on twelve days, but the month was very dry with a total rainfall of only 0·48 in., 0·30 in. of this on the 16th.

Control. The removal of the savin from the locality would be an effective means of controlling this rust, or the cutting-out of hypertrophied shoots before they produce teleutospores could be attempted, but in an ornamental garden or a nursery this is not always desirable, and spraying the medlar in spring with Bordeaux mixture (4–6–100) or some other suitable copper preparation would be worthy of trial. Duggar (1909) regarded the spraying of the aecidial host with standard Bordeaux mixture 'at about the time of ripening of the teleutospores' as of some value; Grove (1913), however, stated that 'it is useless to spray the aecidial host'.

Summary

A severe aecidial attack in 1943 and a slight attack in 1944 of the uncommon Cluster-Cup Rust, *Gymnosporangium confusum* Plowr., on *Mespilus germanica* in a Kent garden are described. Two bushes of a variety of the teleutospore host, *Juniperus Sabina*, were growing nearby.

The writer is indebted to Mr R. V. Harris for his helpful criticism in the preparation of this note, to Mr W. C. Moore for a summary of the literature, to Dr H. Wormald for his helpful interest throughout, and to Mr D. Akenhead for a translation.

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EXPLANATION OF PLATE I

Fig. 1. Medlar Cluster-Cup Rust on leaves and fruit (× approx. ⅔).
Fig. 2. Hypertrophied branch of *Juniperus Sabina* with teleutospore stage of *Gymnosporangium confusum* Plowr. (nat. size). Note revived teleutosorus protruding on left.

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ON THE SPECIALIZATION OF *BREMIA LACTUCAE* ON COMPOSITAE

By LEE LING and M. C. TAI, *Chengtu, China*

(With Four Text-figures)

Introduction

The genus *Bremia* was established by Regel in 1843 for the single species *B. Lactucae*, which has remained the best-known species of the genus and has been reported from widely separated regions. In addition to *Lactuca*, members of the following genera of Compositae have been recorded as host plants for *Bremia*: *Carduus*, *Carthamus*, *Centaurea*, *Cichorium*, *Cirsium*, *Conyza*, *Crepis*, *Cynara*, *Gaillardia*, *Gnaphalium*, *Hieracium*, *Krigia*, *Lappa* (*Arctium*), *Lapsana* (*Lampsana*), *Leontodon*, *Picris*, *Prenanthes*, *Senecio*, *Sonchus*, *Taraxacum*, *Tragopogon*. Schweizer (1919) concluded, from the results of his observations and experiments, that *Bremia Lactucae* includes a number of biologic forms which are specialized to distinct hosts and may be separated to some extent on biometrical data. Sydow (1923, 1938), following Schweizer, separated three of these forms as distinct species, namely, *B. Tulasnei* (Hoffm.) Syd. (from *Senecio*), *B. Centaureae* Syd. (from *Centaurea*) and *B. Lampsanae* Syd. (from *Lapsana*). Sawada (1919) named five species from Formosa, namely, *B. elliptica* Saw. (from *Lactuca laciniata* Makino = *L. indica* L. and *L. formosana* Maxim.), *B. microspora* Saw. (from *Lactuca debilis* Mass.), *B. sonchicola* (Schlecht.) Saw. (from *Sonchus oleraceus* L.), *B. ovata* Saw. (from *Crepis japonicus* Benth.), and *B. Saussureae* Saw. (from *Hemistepta carthamoides* O. Kuntze = *Saussurea affinis* Spreng.). Ito (1938) listed a few additional hosts for *B. elliptica*, *B. microspora* and *B. sonchicola*. Ito and Tokunaga (1935) had already described two new species from Japan, *Bremia Taraxaci* and *B. Picridis*. Milovtzova (1937), working in the Ukraine, held the mildew on *Carthamus tinctorius* L. to be a new form, *Bremia Lactucae* Regel f. *Carthami* Milovtzova. Jagger and Chandler (1933) and Schultze and Röder (1938) demonstrated the existence within *Bremia Lactucae* of a number of physiologic races by showing that the various forms differed in their pathogenicity towards varieties of the cultivated lettuce.

Between 1942 and 1944, *Bremia* has been collected in the vicinity of Chengtu, China on the following seven host plants: *Lactuca sativa* (leaf type), *L. indica*, *L. chinensis* Makino, *Crepis japonicus*, *Sonchus oleraceus*, *Taraxacum mongolicum* Hand.-Mzt., and *Saussurea affinis*. Except for the fungus from *Lactuca chinensis*, these collections of *Bremia* had already been recorded and assigned to six distinct species. As it was possible to obtain all these forms in abundance, there was an excellent opportunity for a comparative study on which the taxonomic relationships of the forms could be ascertained.

Comparative morphology

Details of the morphology of *Bremia* collected from the seven hosts are given in Table 1; Figs. 1 and 2 illustrate the results of five hundred measurements of the lengths and widths of the sporangia of the seven collections. All observations were taken from fresh material mounted in lacto-phenol or, rarely, in distilled water. A study of Table 1 and of the corresponding text-figures shows that the collections of *Bremia* from the seven hosts can be arranged in three groups. The fungus from *Saussurea* is distinguished by its large sporangia, and the measurements from this material overlap very little with those from the other gatherings. The fungi from *Lactuca chinensis* and *Crepis japonicus* have small sporangia, and the rest form a third group with sporangia of medium sizes.

The shape of the sporangium, taken by itself, is not a reliable guide for the separation of species. In a single gathering, sporangia ranging in shape from subglobose to elliptical may be found (Fig. 3). Sawada applied the specific epithet *ovata* to the *Bremia* on *Crepis japonicus*, but ovate sporangia may be found in greater or less numbers in most gatherings. The length/width index shows that the sporangia of the fungus from *Crepis japonicus* are more elongated than sporangia taken from other host plants, but the difference is not great enough to make it possible to separate the various forms by microscopic examination.

The *Bremia* from *Crepis japonicus* has very long sporangiophores, ranging from 850 to 1050 μ in length; this character agrees with the original description of *Bremia ovata* from the same host. The other characters of the sporangiophores, such as the method of branching, and the proportion between the lengths of the whole sporangiophore and of the unbranched portion, characters which were emphasized by Sawada (1919), are too variable to be used for taxonomic purposes. In their morphology, the fungi from *Lactuca sativa* and *L. indica* agree more closely with one another than with those from the other hosts.

Inoculation experiments

Between December 1942 and February 1944, many cross inoculations were made. The inocula were obtained, in part from naturally infected hosts growing in the open, and in part from artificially infected hosts in the laboratory. The inoculations were made by spraying healthy host plants with suspensions of sporangia in water. Satisfactory results were obtained by using water rich in minerals, and especially in salts of calcium and magnesium, taken from a well; it had already been found that the sporangia germinated better in this well water than in rain water, river water or distilled water. The poorest germinations were obtained in distilled water, which gave germination percentages of 23 % after 128 hours and of 36·7 % after 176 hours; the corresponding figures for well water, river water and rain water were: after 128 hours, 62·8, 45·4 and 34·2 %; after 176 hours, 63·1, 60·7 and 51·6 %.

Table 1. *Morphology of* Bremia *on seven species of Compositae*

Host	Morphology of sporangiophores: Sporangiophore growth	Times of branching	Length in μ	Width in μ	No. of sterigmata	Morphology of sporangia: Average shape	Length* in μ: Range	Length* in μ: Mean	Width* in μ: Range	Width* in μ: Mean	Length/width
Saussurea affinis	Amphigenous, dense	4–7	206–650	7·6–11·4	3–6	Broadly elliptic	22·9–37·2	30·37 ± 1·120	20·0–32·9	27·77 ± 0·908	1·09
Lactuca sativa	Mostly hypophyllous, dense	3–6	275–420	11·4–15·2	3–5	Subglobose	10·0–24·3	17·61 ± 1·073	10·0–22·9	16·26 ± 0·807	1·08
L. indica	Mostly hypophyllous, dense	4–6	425–610	7·6–14·3	4–7	Subglobose	11·4–24·3	17·50 ± 0·982	11·4–22·9	16·40 ± 1·028	1·06
L. chinensis	Hypophyllous, sparse	4–6	310–510	9·5–13·4	3–5	Subglobose	10·0–21·5	15·32 ± 0·897	8·6–20·0	14·05 ± 0·851	1·09
Crepis japonicus	Hypophyllous, sparse	5–8	850–1050	7·6–11·4	4–7	Ovate to elliptic	10·0–21·5	15·63 ± 0·971	7·2–19·2	11·89 ± 0·777	1·31
Taraxacum mongolicum	Hypophyllous, sparse	4–6	428–545	9·5–13·4	3–6	Broadly elliptic	12·9–24·3	18·54 ± 0·886	11·4–21·5	16·49 ± 0·882	1·12
Sonchus oleraceus	Hypophyllous, dense	3–6	332–534	11·4–13·4	3–6	Broadly elliptic	12·9–24·3	18·54 ± 0·943	8·6–21·5	16·12 ± 1·066	1·14

* Based upon 500 measurements

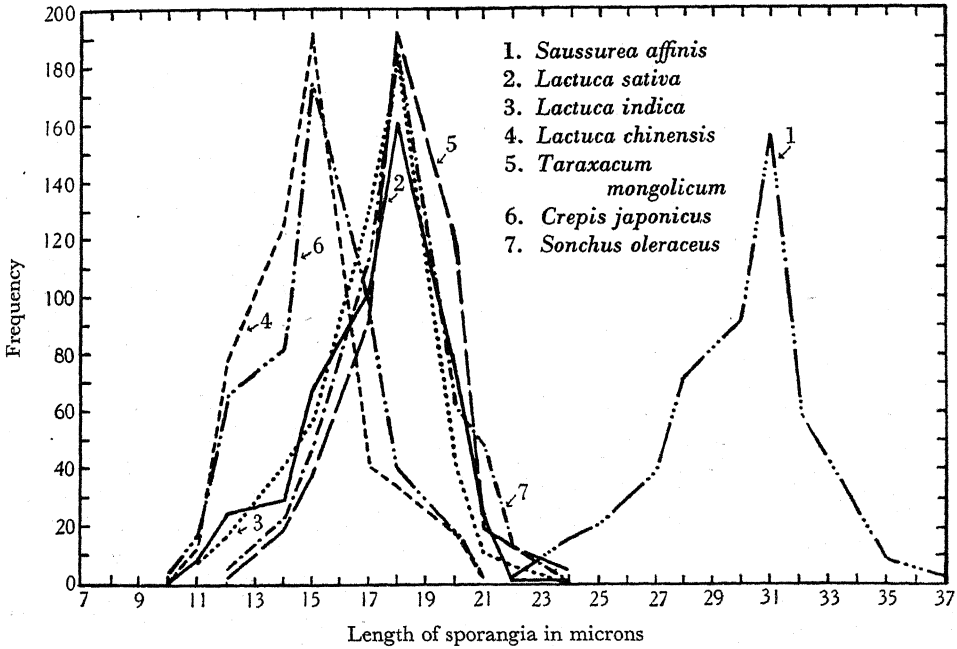

Fig. 1. The distribution in length of 500 sporangia of *Bremia* on seven hosts.

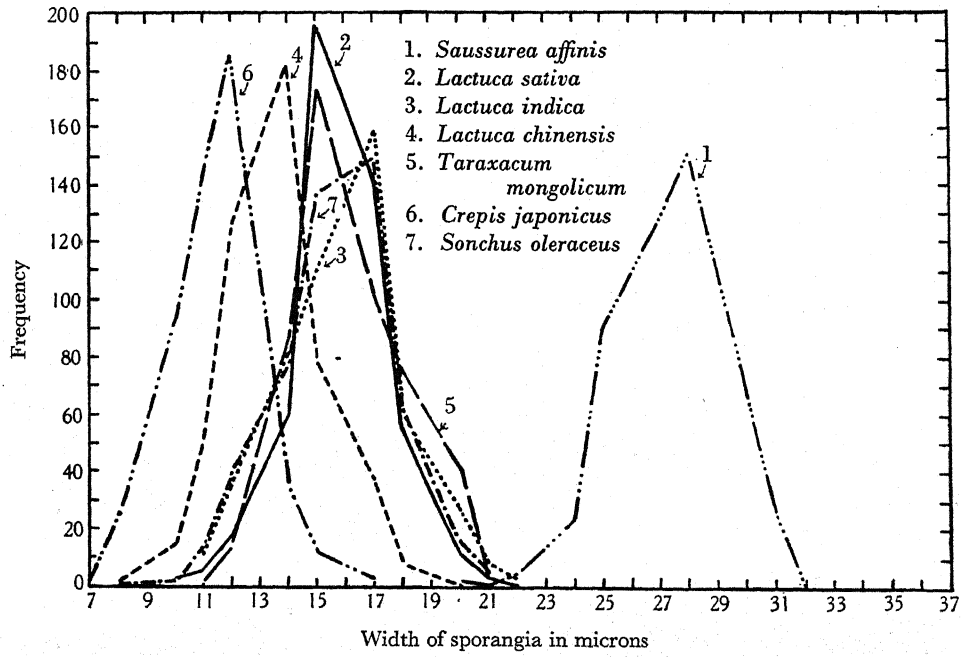

Fig. 2. The distribution in width of 500 sporangia of *Bremia* on seven hosts.

After the host plants had been inoculated they were kept in a moist chamber for two to three days; detached leaves were also inoculated to give a check on the experiments with growing plants. If the host was susceptible to the fungus, sporangiophores appeared in from nine to twelve days after inoculation. The results of the experiments are summarized in

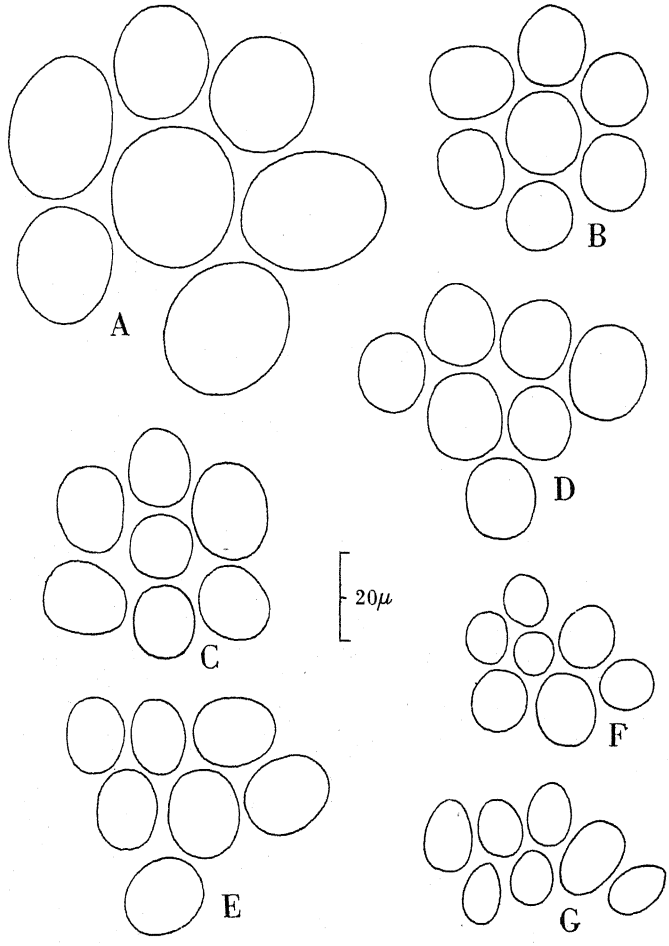

Fig. 3. Sporangia of *Bremia* on seven hosts. A, *Saussurea affinis*. B, *Lactuca sativa*. C, *Lactuca indica*. D, *Sonchus oleraceus*. E, *Taraxacum mongolicum*. F, *Lactuca chinensis*. G, *Crepis japonicus*.

Table 2. They show that cross-inoculation is possible between *Lactuca sativa* and *L. indica*, but that the forms of *Bremia* from the other hosts are confined to their respective hosts. *Bremia* from *Lactuca chinensis* will not infect *L. sativa* or *L. indica*. These findings are considered to show that immunity or susceptibility to a parasite gives good information about the relationships of the host plants. In both *Lactuca sativa* and *L. indica* the

basic chromosome number is 9, and cross-inoculation succeeds; *L. chinensis* has a basic number 16, and on this, and on the reactions to *Bremia*, *Lactuca chinensis* appears to be but remotely related to the other two species of *Lactuca*. Schultze and Röder (1938) reported that *L. scariola* and *L. virosa* react to infection by downy mildew much as did the cultivated lettuce; these two species belong to the 9-chromosome group.

Table 2. *Results of cross-inoculation with* Bremia *on seven species of Compositae*

Source of inoculum	*Lactuca sativa*	*Lactuca indica*	*Lactuca chinensis*	*Taraxacum mongolicum*	*Crepis japonicus*	*Saussurea affinis*	*Sonchus oleraceus*
Lactuca sativa	+	+	−	−	−	−	−
L. indica	+	+	−	−	−	−	−
L. chinensis	−	−	+	−	−	−	−
Taraxacum mongolicum	−	−	−	+	−	−	−
Crepis japonicus	−	−	−	−	+	−	−
Saussurea affinis	−	−	−	−	−	+	−
Sonchus oleraceus	−	−	−	−	−	−	+

+ infection; − no infection.

Factors affecting the size of the sporangia

Schweizer (1919) showed that the dimensions of the sporangia of *Bremia Lactucae* are influenced by the host plant as well as by humidity. As some taxonomists have emphasized the importance of biometric data in dealing with fungi, it seemed worth while to determine the range of variation in the dimensions of sporangia in relation to environmental conditions, and to consider the value of the data to the taxonomist.

For this purpose, *Bremia* was grown on the cultivated lettuce; attention was paid to the effect of temperature, age, and the nature of the host tissue on the dimensions of the sporangia. The effect of temperature was tested by measurements taken from sporangia grown at 10° C., and at 24–28° C. Detached leaves, after being thoroughly washed to remove any sporangia then present on them, were placed in moist chambers for eighteen and for thirty-six hours, and the resulting sporangia sampled and measured. The influence of the host tissue was investigated by comparing the dimensions of sporangia grown on cotyledons and on mature leaves respectively. The results of all these tests appear in Table 3. The measurements (when allowance is made for probable error) show that both temperature and age have significant effects upon the lengths and widths of sporangia, and that there is very little difference between sporangia grown on cotyledons and on mature leaves of the host.

Ecological relations

The behaviour of *Bremia* on the seven hosts in the field was not uniform, for there were differences in developmental history, in the date of appearance and in the duration and extent of infection. These differences were not entirely due to the association of the fungus with the growing season of the host, and it seems likely that there was some difference in the

response of the fungi to environmental conditions. Lettuce is cultivated in the neighbourhood of Chengtu for almost the whole of the year, and its mildew has the longest period of occurrence, generally lasting for ten months, from October to July. A few of the other hosts, *Lactuca chinensis*, *Crepis japonicus* and *Taraxacum mongolicum* are present for almost all the year, except for a short time in the hottest part of the summer, and yet on them *Bremia* is limited in occurrence from late autumn to early spring. *Sonchus oleraceus*, *Saussurea affinis* and *Lactuca indica* are relatively short-lived and therefore are infected for but a short period. Throughout 1943, observations were made every ten days, so that the first and last appearances

Table 3. *Factors influencing the dimensions of sporangia of* Bremia Lactucae *on cultivated lettuce*

	Temperature		
Measurement	10° C.	24–28° C.	Difference
Length: Range	11·4–24·3	10·0–21·5	
Mean	17·95 ± 0·705	16·37 ± 0·795	1·58 ± 0·079*
Width: Range	11·4–22·9	8·6–21·5	
Mean	16·58 ± 1·187	15·17 ± 1·036	1·41 ± 0·111*
Length/width	1·08	1·08	
	Age of sporangia		
Measurement	18 hr.	36 hr.	Difference
Length: Range	8·6–20·0	11·4–21·5	
Mean	14·82 ± 0·820	16·57 ± 0·818	1·75 ± 0·081*
Width Range	8·6–20·0	10·0–21·5	
Mean	14·14 ± 0·851	15·69 ± 1·005	1·55 ± 0·090*
Length/width	1·05	1·12	
	Host tissue		
Measurements	Mature leaf	Cotyledon	Difference
Length: Range	11·4–24·3	11·4–24·3	
Mean	17·95 ± 0·705	17·59 ± 0·940	0·36 ± 0·083*
Width: Range	11·4–22·9	11·4–22·9	
Mean	16·58 ± 1·187	16·65 ± 0·762	0·07 ± 0·101
Length/width	1·08	1·06	

* Significant ($D./P.E._d > 3·2$).

of the fungi could be determined; the results of these observations are given in Fig. 4. Usually, *Bremia* is not to be found during the hot season; in July, August and September, though relative humidity is usually at or above 80 %, the high temperature checks the growth of *Bremia*. Very occasionally, during the hot season, the mildew may be found in shady places where the temperature is relatively low and humidity higher than in the open field; once, in August 1943, a heavily infected lettuce was found under a hedge.

As the mildew disappears in the hot season, its manner of over-summering comes into question. Most mildews are narrowly specialized to certain hosts, and it seems very unlikely that there are summer hosts. Weber's

suggestion (1928) that wild plants act as centres of infection from which the mildew spreads to the crops can hardly be accepted. *Bremia* rarely forms oospores and the sporangia seldom survive long enough to serve as sources of infection in the autumn. Angell and Hill (1931) stated that sporangia of *Bremia Lactucae* from lettuce gave 1 % germination after desiccation for thirty-five days. At Chengtu, the high humidity and high summer temperatures greatly reduce the viability of the sporangia; even in March, very few sporangia were alive in a sample taken from cultivated lettuce and exposed on dry slides in the laboratory for 100 hours. If this happens with *Bremia* from lettuce it will be even more difficult for over-summering by sporangia to occur in *Bremia* from other hosts, with a shorter growing season and a longer gap in the life cycle to be covered. The

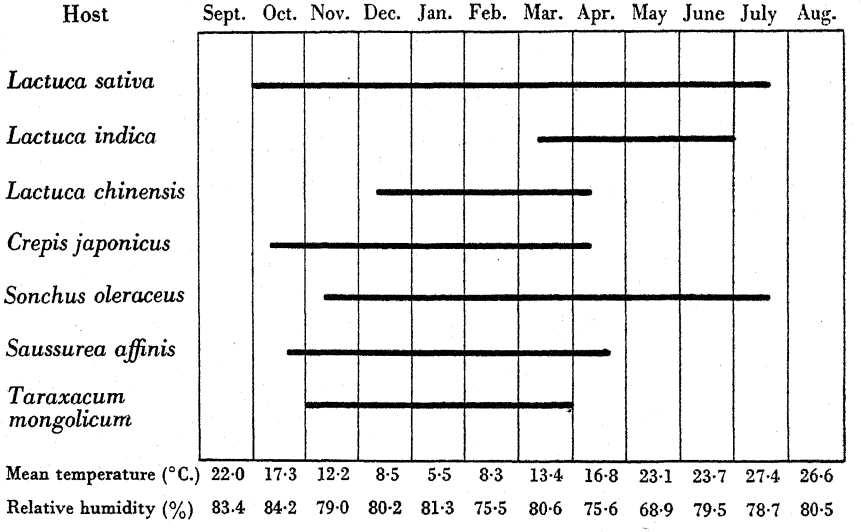

Fig. 4. Seasonal occurrence of *Bremia* on seven species of Compositae in Chengtu, 1943.

occasional appearance in infection on seedlings of lettuce germinated in Petri dishes suggests that these mildews very probably over-summer in infected seeds.

Centaurea Cyanus, *Picris hieracioides* and species of *Cirsium* are rather common around Chengtu, and have been reported as hosts of *Bremia* in other parts of the world, but prolonged search has failed to reveal *Bremia* on these plants at Chengtu.

Discussion

Since Gäumann (1923) published his work on *Peronospora* there has been a widespread tendency to separate the species belonging to this family on very small characters. A number of old and well-known species have been split on biometric data and on specialization to certain host plants, and usually this has been done without experimental evidence. *Bremia Lactucae*

has been recorded on species belonging to twenty-two genera of the Compositae. If all the forms occurring on different genera or even on different species should be proved to be specialized in their parasitism, is it desirable to establish at least twenty-two species to accommodate these forms? If that is done, there will be a great multiplication of names, and taxonomic work will be made very difficult. Moreover, environmental conditions may affect the morphology of the fungus as greatly as does a difference of host. The present work shows that age and temperature can cause statistically significant differences in the dimensions of the sporangia, and further evidence of variation due to environmental conditions is provided by the fact that the published descriptions of these fungi do not agree completely with our observations, even though the hosts are the same.

To satisfy the general requirements of taxonomy, species should be based on readily distinguishable characters. The meaning of the words 'readily distinguishable' is all important, but there is no general agreement about that, and disputes are therefore frequent. As a result of this study of *Bremia*, the fungus from *Saussurea* is considered to be a distinct species because it is easily distinguished from all the others by its large sporangia. Its host plant, *Saussurea*, belongs to the tribe Cynareae of the Compositae, and is widely separated in its relationships from the six other hosts, all of which belong to the Cichorieae; for the fungus from *Saussurea*, the name *Bremia Saussureae* Sawada is accepted. The fungi from the other six hosts are all held to be forms of *Bremia Lactucae* Regel; some show marked specialization to one host, and they may also be separated by morphological characters which are not however very marked. The fungus from *Crepis japonicus* with its long sporangiophores and a tendency to form a preponderance of narrow sporangia, may deserve specific rank, but for the present it is regarded as a form of *Bremia Lactucae*.

The following revision of nomenclature is proposed:

Bremia Lactucae Regel (syn. *B. elliptica* Saw.). On *Lactuca sativa* and *L. indica*.

Bremia Lactucae Regel f. **chinensis** f.n. On *Lactuca chinensis*; forma specialis ex *Lactuca chinensis*; sporangia parvis.

Bremia Lactucae Regel f. **sonchicola** (Schlecht.) comb.nov. (syn. *Botrytis sonchicola* Schlecht.: *Bremia Sonchi* Saw.). On *Sonchus oleraceus*.

Bremia Lactucae Regel f. **Taraxaci** (Ito & Tokunaga) comb.nov. (syn. *Bremia Taraxaci* Ito & Tokunaga). On *Taraxacum mongolicum*.

Bremia Lactucae Regel f. **ovata** (Saw.) comb.nov. (syn. *Bremia ovata* Saw.). On *Crepis japonicus*.

Summary

Bremia was collected near Chengtu, China, from seven genera of Compositae, and the forms from these hosts can be placed in three groups, based on the dimensions of the sporangia. The fungus from *Saussurea* is regarded as a distinct species, *Bremia Saussureae* Saw., the others as *Bremia Lactucae* and forms of that species. Successful cross-inoculations were made between *Lactuca sativa* and *L. indica*, but the other fungi were specialized to their natural hosts.

The taxonomy of *Bremia* is reviewed, and a revision proposed.

Experiments showed that the dimensions of the sporangia could be influenced by temperature and by age. It is suggested that *Bremia* survives the hot part of the summer, when high temperatures prevent its growth, by means of infected seeds.

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THE DISPERSION OF AIR-BORNE SPORES

By P. H. GREGORY, *Rothamsted Experimental Station, Harpenden, Herts*

(With 6 Text-figures)

I. Introduction

The dispersal of passively air-borne particles, such as seeds, spores or pollen, results in a *scatter* around the point of liberation. The process is influenced by many factors, so unpredictable at first sight that the resulting dispersion pattern might appear to defy analysis. An analysis should be attempted, however, because knowledge of the pattern is fundamental for the understanding and control of the dispersal process itself. The discussion that follows will be based mainly on dry fungus spores in the role of plant pathogens, but it applies to other particles carried passively by wind, such as seeds, pollen (microspores) and respiratory allergens, and has a bearing on the dispersal of some viruses and mutant genes.

Certain facts about the process are widely recognized. The practice of isolating clean plants and crops from sources of disease or foreign pollen depends on the observed fact that the amount of contamination suffered generally decreases the farther away one gets from the source. This was recognized for cereal diseases by Windt (1806, cited from Ramsbottom, 1934), who found that rust on rye was severe near barberry bushes, which are now known to be the alternate host of the fungus: 'These effects are striking and desolating in the distance of ten to twelve paces. I have also perceived them visibly at the distance of 50, 100, 150 paces' and a final attack at 'above 1000 paces'. The scatter pattern is thus recognized as producing, in graphical representation, a gradient of infection. This idea underlies Pape and Rademacher's (1934) proof that in Germany, winter barley contaminates the spring-sown crop with *Erysiphe graminis*, and Bonde and Schultz's (1943) demonstration that in Maine, potato-refuse piles are the principal sources of the contamination of potato fields with *Phytophthora infestans*. The problem is to find whether any general relation exists between these gradients. The problem is statistical because, while the destination of a single spore in a wind eddy is inscrutable, the average distribution of vast numbers of spores over a uniform area and over a period of time offers hope of rational treatment.

Brief mention must first be made of some earlier attempts to formulate such a relationship on abstract grounds. Nägeli (see Stepanov, 1935) concluded that the density of dust particles should fall off inversely as the square of the distance from the source. Kursanov (see Stepanov, 1935) stated that in the absence of wind the number of fungus spores would fall

off inversely as the cube of the distance from the source. Without any proof or reference to other authors, Fischer and Gäumann (1929) state that with linear increase of the distance the chances of infection by rust spores decrease in cubic progression. These formulations appear to rest on the assumption that a passively air-borne particle can be treated like a radiation, passing outwards into space in a straight line until obstructed. However, since our particles are air-borne they cannot radiate in this manner because the air itself is obviously not in process of being continuously generated at all points in the atmosphere and consequently some totally different concept is needed.

Qualitative aspects of the dispersal of fungus spores in air are reviewed by Butler (1917), Gardner (1918), Weston (1923), Stepanov (1935), Craigie (1940), Keitt (1942) and Christensen (1942). For pollen, reference should be made to Rempe (1937) and to Erdtman (1943). The role of air-borne moulds in asthma is reviewed by Durham (1942) and by Morrow and Lowe (1943).

This survey first discusses the nature and magnitude of factors that control the scattering of air plankton. The literature is then examined to see whether the observed distributions of plant pathogens follow any recognizable patterns. Finally, the application of the subject is discussed with special reference to the study and prevention of air-borne diseases of field crops.

II. The factors controlling dispersal

The first stage in dispersal is the liberation of spores or pollen from the structures in which they were formed. The mechanisms of spore discharge in the fungi are the subject of an extensive series of researches by Buller (1909–34). Ingold (1939) has given a concise account of spore discharge in land plants, while Dobbs (1942) gives a classification of spore-dispersal mechanisms in the fungi. The mechanisms for liberating pollen are dealt with by Kerner and Oliver (1895), and more recently by Rempe (1937). After liberation from the plant the spore is operated on by various forces such as gravity, wind currents and electrostatic forces. It will be convenient to consider first those forces acting on a single particle and then to discuss work relevant to groups of spores.

(1) *The fate of an individual spore.* In still air, spores fall slowly under gravity. The open air, however, is never still, and even inside a closed room spores can be carried in feeble air currents and deposited far from the point of liberation. However, the rate at which spores fall in still air has been studied as a factor in distant dispersal and must be considered.

The period after discharge during which a basidiospore, for instance, is undergoing a positive acceleration due to gravity is very short on account of the small mass of the spore in comparison with its area. After a fall of less than one diameter of the spore (10 μ) of *Amanitopsis vaginata*, Buller (1909) found that acceleration was balanced by increased air resistance and that the fall continued thereafter at a constant terminal velocity. It is likely that this velocity even decreases if desiccation further reduces the

mass of the spore. Stokes's law described a relation between the size and velocity of fall of a smooth sphere in a viscous fluid in the following terms:

$$V = \frac{2}{9} \cdot \frac{\rho - \sigma}{\mu} g r^2,$$

where V = terminal velocity, ρ = density of the sphere, σ = density of the medium, g = acceleration due to gravity, r = radius of the sphere, μ = viscosity of the medium.

Using as a test object the nearly spherical spores of *Amanitopsis vaginata*, with a density in moist air of 1·02, Buller (1909) observed terminal velocities about 50% greater than expected from Stokes's law. Later (Buller, 1922) this was attributed to the effective size of the spore being increased by a droplet of water which was always found to be excreted and carried away when the spore was discharged from the basidium. Further work showed that on leaving the fungus fruit-body and falling through dry air the spores rapidly lost velocity owing to desiccation, and completely air-dried spores were seen to fall only one-third as fast as fully turgid spores.

That Stokes's law adequately described the fall in air of artificial spheres of wax, mercury, etc., of the same order of magnitude as fungus spores, has also been shown by Zeleny and McKeehan (1910), but they found that spores of *Lycopodium*, *Bovista* and *Polytrichum* fall only about half as fast as predicted by the theory. This they attributed to irregularities on the surface of the spores setting up eddies in the immediate neighbourhood of the particle, while Stokes's law applies to non-turbulent conditions. Rempe (1937) has suggested that, because of surface irregularities, pollen grains may become coated with a stationary surface layer of air which increases their effective diameter.

Exact prediction of the terminal velocity of a spore is hindered by the difficulty of determining its density (which in turn depends on its hydration), and by deviation from the ideal smooth spherical form. In Fig. 1. are plotted the terminal velocities of spores and pollen grains observed by various workers, together with the velocity to be expected from Stokes's law for a sphere of density 1·00. The sizes of asymmetrical spores are represented on the graph as the geometric mean of the short and long axes. Assuming $\rho = 1{\cdot}00$, σ negligible, $\mu = 1{\cdot}8 \times 10^{-4}$ (viscosity of air), $g = 981$ in c.g.s. units, we have $V = 0{\cdot}01211r^2$, where r is the *radius* of the sphere in microns, and V is in cm. per sec.

According to Erdtman (1943), the observed terminal velocities of air-dry pollen are usually somewhat higher than the expected values. Pohl (cited from Erdtman) found that the specific gravity of pollen grains varied from 1·161 for *Typha latifolia* down to 0·391 for *Pinus sylvestris*. Buller (1909) found the density of hydrated spores of Agaricaceae to be about 1·02, while for spores of *Polytrichum*, Zeleny and McKeehan (1910) recorded a value as high as 1·53. In general, the observed terminal velocities of pollen are greater than those of fungus spores, varying from nearly 40 cm.sec. for *Abies*, down to about 1·5 cm.sec. for *Alnus viridis*. Fungus spores tend to fall more slowly, and vary from nearly 3·0 cm.sec. for *Helminthosporium*

sativum, down to 0·05 cm.sec. for species of *Lycoperdon*. The biggest deviations from expectation are shown by the slow-falling pollen of *Picea* and *Pinus*, which have large air sacs, and by the relatively fast-falling caudate spores of *Bovista plumbea*. In general, it may be said that the terminal velocity of roughly spherical spores is of the order expected from Stokes's law (Fig. 1).

McCubbin (1918), Ukkelberg (1933) and Stepanov (1935) allowed spores of rusts and other fungi to fall in columns of air in vertical cylinders,

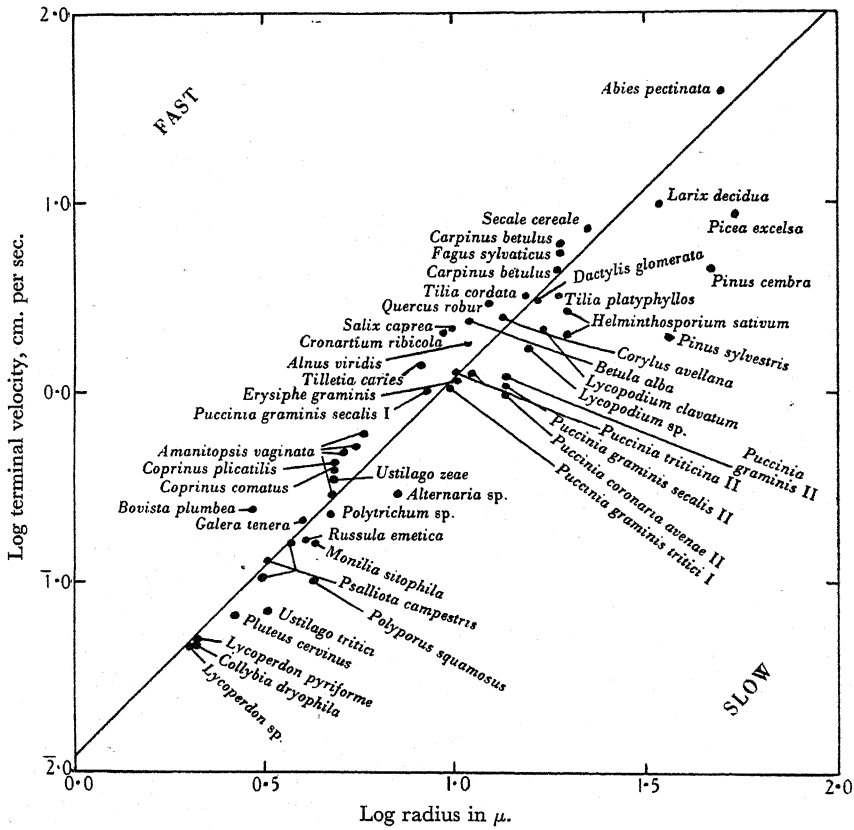

Fig. 1. Observed terminal velocities of fall of spores and pollen grains in cm. per sec., plotted against radius in microns (if highly asymmetric the geometric mean of long and short axes is taken) from data of Buller, Zaleny and McKeehan, McCubbin, Ukkelberg, Stepanov, Rempe, Yarwood and Hazen, Christensen, and Erdtman. The straight line represents expected velocity of fall of a smooth sphere of density = 1·00, according to Stokes's law.

and deduced the terminal velocity from the numbers of spores deposited at the foot of the column at successive intervals after liberation of the spores. In general, both McCubbin and Ukkelberg obtained data of a similar type, and a graph of the number of spores reaching the bottom of the tube in successive time intervals showed a negative skew distribution.

Part of the skewness of the curve was shown by Ukkelberg to be due to clumps of spores falling with a high velocity, but it is equally clear that uredospores and aecidiospores of rusts also show skewness due to an excess of single spores falling very slowly. Unfortunately, neither McCubbin, Ukkelberg nor Stepanov measured the spores to find whether the first to arrive at the bottom of the column of air were larger than those arriving at the end of the run, and the effect may have been due to an experimental error caused by eddies in the column of air. Ukkelberg showed that differences in the rate of spore fall of uredospores and aecidiospores of *Puccinia graminis tritici* and *P.g. secalis* were probably due to differences in size, but it remains to be shown whether differences in the terminal velocity within a population are also due to differences in size. Ingold (1939), studying the violently discharged spherical spores of *Conidiobolus villosus* whose diameters vary from 15 to 48 μ, found that in still air, as might be expected from Stokes's law if the spores were all shot off with the same initial velocity irrespective of size, the spores were discharged to horizontal distances proportional to the square of their radius.

Stokes's law applies only to smooth spherical particles. Most fungus spores are not isodiametric but more or less elongated; the ascospores of *Ophiobolus graminis* for instance have a length about twenty times their width. Buller (1909) observed that elongated spores tend to fall horizontally and that the final position which the spore took up in air was, as would be expected, such that the greatest surface was presented to the resistance of the air. Under experimental conditions, however, *Erysiphe graminis* with conidia measuring $32 \times 20\,\mu$ was found by Yarwood and Hazen (1942) to behave anomalously.

For a spore of a given volume any elongation will expose a relatively larger surface and so decrease the rate of fall to a value below that expected for a sphere. Very few tests appear to have been made on the terminal velocity of markedly asymmetrical spores, interest having been focused on spherical particles by Stokes's law. McCubbin (1944) deals with the fall of non-spherical spores and stresses the need for more empirical data with precautions against eddies. The fall of *Helminthosporium sativum* has been studied by Stepanov (1935) and Christensen (1942) who obtained values of 2·78 and 2·0 cm.sec. respectively, for spores measuring $68 \times 24\,\mu$ and $75 \times 20\,\mu$. It can be calculated that a cylinder of such dimensions would approximately equal the volume of a sphere of 40 μ diameter with an expected terminal velocity of about 4·8 cm.sec. Christensen also tested spores of *Alternaria* sp. $20 \times 10\,\mu$, and observed a speed of 0·3 cm.sec. This, it may be suggested, is nearer the speed one would expect if the spores fell with their long axes vertical, as some *Alternaria* spores might do owing to the feathering effect of the elongated beak of the spore. I have observed that the caudate spores of *Bovista plumbea* are orientated in this way during fall. More observational data are evidently needed before it can be assumed that all elongated spores fall more slowly than would spheres of the same volume.

The effect of one of the forces acting on spores in air can now be summarized. The vertical effect of gravity results in a terminal velocity

of fall of the order of 1 cm.sec. According to the size and shape of the spore the speed is observed to vary from 0·05 cm.sec. for *Lycoperdon* to 2·8 cm.sec. for *Helminthosporium sativum*, and 9·0 cm.sec. for pollen of *Picea excelsa*. Within an individual species the speed may vary by 100%, as with Ukkelberg's observations on *Puccinia graminis secalis* (0·73–1·47, with a mean of 1·02 cm.sec.), possibly owing to variations in the size of individual spores. Elongated spores sometimes do and sometimes do not fall slower than would spherical particles of the same volume, possibly because of mechanisms affecting orientation.

(2) *Minor forces acting on spores*. The basidiospores of the mushroom and some other fungi have been shown by Buller (1909) to carry small electric charges when falling in air. Little is known about the phenomenon, and its effect is probably negligible except when the spore is within about a millimetre of another body. The origin of the charge, its effect over very short distances, and its relation to the vertical potential gradient in the atmosphere, which is said to average about 150 V. per m., might repay future investigation. It is also known that small particles tend to move down a temperature gradient (Cawood, 1936; Watson, 1936), and that forces exist causing particles, at least up to 2 μ in diameter, to be repelled by a hot surface and attracted by a cold one. Smoke particles and *Lycopodium* spores are also known to move away from light (photophoresis, Whytlaw-Gray & Patterson, 1932).

(3) *Wind*. Besides the vertical force of gravity, spores are also acted on by wind which can be considered as a force acting horizontally. The mean velocity in England is considered to be about 5 m. per sec. (Sutton, 1932). Wind records usually refer to a standard anemometer exposed at 10 m. above the ground. Wind velocity tends to increase with height, and the speed at 2 m. would be only about 0·78 of that at the standard height. Summaries of wind-frequency data in the detail required for understanding the present problem are seldom given, but tables of wind frequency at 7 h. and 18 h. for Lerwick, Shetland, and for Scilly, England, each based on 3625 observations, are given by Bilham (1938). At both these coastal stations over 96% of the observations showed winds above 0·3 m.sec. The highest frequency class was 3·4–5·4 m.sec. at Lerwick, and 5·5–7·9 m.sec. at Scilly. As an example of wind velocity frequencies at an inland station the hourly readings for the year 1937 for the anemometer at Kew, England, have been tabulated from data published by the Meteorological Office (1939). It was found that at Kew nearly 90% of the hourly means were 1·0 m.sec. and upwards, and that for over half the time the speed was 3·0 m.sec. and upwards. Wind speeds therefore are commonly 300 times, and usually at least 100 times as great as the rate of fall of spores under gravity, so it is likely that wind velocity is a major factor in controlling spore dispersal. Wind speed may also play a part in altering the number of spores or pollen grains liberated by the plant, as was realized by Stepanov (1935). Many Hymenomycetes shed their spores irrespective of wind conditions, but others, like puff-balls and probably many rusts and dry-spored *Fungi imperfecti*, liberate more spores the greater the speed of the wind. Many flowering plants have mechanisms which ensure that

pollen is not liberated into air moving at less than a critical speed (Kerne & Oliver, 1895).

(4) *Spore dispersal as the resultant of horizontal and vertical forces.* Previous estimates of the probable limits of spore dispersal have usually been based on the trajectory of an individual spore considered as the resultant of its vertical fall under gravity and its horizontal transport by wind. Data on the slow speed of fall have been quoted as evidence that spores may be carried for great distances.

McCubbin (1918) considered that 'the general problem of spore dispersal is vitally a question of maximum dispersal distance', a view that is contested in this paper. He accordingly concentrated most attention on the relatively few spores in his experiments, described above, which fell most slowly and were therefore likely to be carried farthest by air currents. It was calculated that spores falling at 0·8 cm.sec., and liberated from a height of 8 ft. would be carried by a 30 mile per hour breeze for $2\frac{1}{2}$ miles before settling. It was realized that deflexions of the wind and rising masses of heated air would often tend to carry the spores upwards and thus increase the dispersal distance (see Buller, 1924, p. 560). Similarly, Christensen (1942) calculated the approximate theoretical dispersal distance of spores falling under gravity from a height of 1 mile in a 20 mile per hour wind, and estimated that an *Alternaria* spore would travel 2900 miles before reaching the ground.

Table 1. *Percentage of total number of spores trapped at various distances from the point of liberation of a mixed cloud of* Tilletia *and* Bovista (from data by Stepanov, 1935)

	Distance in metres			
Species	5	10	20	40
Tilletia caries	74·1	17·8	7·4	0·7
Bovista plumbea	74·4	19·9	4·9	0·8

Such estimates are based on highly artificial conditions in which the spore is supposed to be raised to a given height and then dropped through a steady stream of air moving horizontally. Stream-line conditions do not as a rule extend more than a millimetre or two above the surface. There is some experimental evidence that the size of a spore and the velocity of its fall have little effect on the dispersal distance. In an experiment conducted in the open air Stepanov (1935) liberated a mixture of spores of *Tilletia caries* and *Bovista plumbea* whose mean diameters and terminal fall velocities he had previously determined as 17 and 5·6 μ and 1·41 and 0·24 cm.sec. respectively. The spores were trapped on the ground at various distances from the point of liberation and at various angles to the prevailing wind. The experiment is discussed fully below, but data summarized in Table 1 show that the relative density of deposit of spores of both species decreased with increasing distance at almost precisely the same rate, in spite of the fact that spores of *Tilletia* fell about six times as fast as those of *Bovista*. In Table 1 the number of spores trapped at each distance is given as a percentage of the total number of spores of each species trapped. The fact

is that the behaviour of an individual spore is unpredictable with our present knowledge. Further progress cannot be made until attention is turned from the fate of the individual spore to that of a group of spores. The methods cited above omit consideration of a third essential factor in spore dispersal, the turbulence of the air.

(5) *The fate of a group of spores.* The foregoing discussion considered the motion of a spore in terms of a vertical component of the order of 1 cm.sec., and of a larger but more variable horizontal component varying from 30 to 2500 cm.sec., with a mean near 300 cm.sec. These conditions only even approximately describe the motion in the comparatively rare cases of non-turbulent air movement, such as under extreme temperature inversions, or katabatic flow (Cornford, 1938).

Watching the drift of smoke from a bonfire or a factory chimney will convince the observer that wind, instead of being a steady streaming motion, is characteristically turbulent. According to Brunt (1934) there are usually present in the atmosphere large numbers of small-scale eddies whose periods are of the order of 1 sec., and at least two-thirds of the eddying energy is associated with eddies of periods of less than 5 sec. The action of the numerous eddies of varying size on the very numerous spores produced from plant sources makes regularity in the dispersal pattern possible.

Turbulence can be mechanical or thermal (Durst in Giblett, 1932). Mechanical turbulence is caused by friction between the wind and the ground over which it passes, and the gustiness developing depends both on the roughness of the ground and the speed of the wind. Thermal turbulence depends on convection currents set up when short-wave radiation from the sun, passing unabsorbed through the atmosphere, warms the ground which in turn warms the layer of air in contact with it by emitting a longer radiation. Under these conditions there may be a rapid decrease (lapse) of temperature with height in the atmosphere and convection currents are set up leading to turbulent mixing of the lower layers of the atmosphere. In clear weather thermal turbulence is at a maximum soon after midday, while on a clear night thermal turbulence may die away completely if cooling of the ground by radiation leads to an inversion of temperature gradient (an increase of temperature with height instead of the usual decrease), in which there is no tendency for lower layers of air to ascend. For an elementary account of this subject reference should be made to Sutcliffe (1940), Brunt (1942), or for greater detail to Brunt (1934).

The effect of turbulence is that a puff of spores or pollen, as well as being carried along in the general direction of the wind, is continually being diluted as one eddy or another mixes the mass of air in which the spores are suspended with other masses of air. Eddying thus diffuses the spores in all directions and, as time passes, so the density of the spore cloud diminishes. Some visual impression of the magnitude of the effect of eddies in diffusing a cloud may be obtained by watching the increasing width of a smoke trail as it drifts away from a chimney. It will become obvious from the subsequent discussion that the magnitude of this diffusion

is such that its effect on a spore cloud is much greater than the effect of gravity.

(6) *The spore cloud regarded as a suspension.* The diameters of air-borne spores and pollen grains may be taken as varying around 10 and 20 μ respectively. This is approximately the magnitude of the droplets forming cloud and fog (Simpson, 1941, 4×10^{-4} to 2×10^{-3} cm.) which are obviously in a state of suspension and show little tendency to sink until they have aggregated. With knowledge of the small speed of spore fall and the large stirring effect of atmospheric turbulence it is tempting to regard the spore cloud also as a suspension.

Like most other suspensions the spore cloud will tend both to precipitate under gravity and to be stirred up by diffusion. The concentrations of spores at different heights in the atmosphere will be the result of the interaction of these two forces. The numbers of spores and pollen grains occurring at different altitudes have been investigated quantitatively in Canada and Germany, and we can make use of the data to see whether the distributions found correspond with known values for terminal velocity and turbulence. The treatment given here is adapted from Schmidt (1925), who shows that for a stable condition, when the number of particles falling under gravity across any horizontal boundary is compensated for by the number of particles moved upwards by diffusion, the concentration of particles in the air decreases exponentially with increasing height according to the equation

$$S = S_0 e^{-Vz/K} \quad \text{or} \quad \log_e S_0/S = V(z/K),$$

where S_0 = concentration at height $z = 0$, V = terminal velocity of fall, K = Taylor's 'eddy diffusivity' constant (assumed invariable with height) and used here in place of Schmidt's 'Austausch' coefficient, A.

During an epidemic of wheat rust in Manitoba in July and August 1930, Peturson (1931) trapped spores at different altitudes in eight aeroplane ascents. The average numbers of spores caught per square inch of trap surface (presumably with comparable exposure times) were: 1000 ft. 10,050 spores; 5000 ft. 1180 spores; 10,000 ft. 28 spores; and 14,000 ft. 11 spores. By substitution in the previous equation we can calculate K, and taking extreme heights find $K = 5{\cdot}8 \times 10^4$, if $V = 1$ cm.sec.

Hubert (1932) trapped spores during two flights at the time of an epidemic of yellow rust of wheat at Halle in Germany. During the second flight, for which data are more extensive, the numbers of spores trapped per square centimetre per minute exposure at various heights were: 30 m. and less, 1418 spores; 400 m. 683 spores; 600 m. 336 spores; and 800 m. 82 spores. Substitution of extreme values in the equation gives $K = 1{\cdot}5 \times 10^4$. The vertical gradient of spore concentration, but with smaller numbers trapped, has also been observed by Stakman and others (1923), Dillon Weston (1929), and Newhall (1938).

According to Brunt (1934, p. 224) meteorological data at heights up to 1000 m. indicate values for K ranging from $1{\cdot}0 \times 10^3$ over the sea during a temperature inversion up to a normal value of 10^5 under average conditions. The distributions of rust spores observed by both Peturson and

Hubert confirm the assumption that spores are present in the air in the form of a stable suspension in which the terminal velocity is balanced by an eddy diffusivity of the usual order of magnitude.

Similar values for K were indicated when tree pollen was trapped over German forests in spring during a series of aeroplane flights by day and by night (Rempe, 1937). For a full discussion of the meteorological conditions prevailing during the individual flights and their effects on the vertical pollen distribution reference should be made to Rempe's original paper. In general, with light to moderate windy weather and with cumulus clouds at about 2000 m., the pollen concentration decreased only slightly up to 1000 m., and the maximum number of grains might occur as high as 200 or even 500 m. This was regarded as a sign of a complete inversion of air masses. A similar distribution also occurred under high-pressure conditions without clouds but with strong thermal turbulence. On the other hand, conditions associated with a stratified cloud layer and high wind velocities showed a marked decrease of pollen with height. In night flights, the maximum number of grains was often reached at a height of about 200 m., that is, above the temperature inversion which often arises at night. Generally there was at night a more pronounced decrease in the numbers trapped at increasing heights than by day. The total numbers trapped at heights were also lower by night than by day.

The mean number of pollen grains trapped per 1·275 sq.cm. of trap surface per 20 min. flight computed from Rempe's data for all flights for which records extended up to 1500 m. was as follows:

Altitude (m.) ...	10–40	200	500	1000	1500
Day flights	904	849	852	581	267
Night flights	577	560	283	85	45

These records include pollen grains of various species, but taking $V=3$ cm.sec. as a moderate value for speed of fall of pollen grains it can be shown that, for altitudes above the region affected by strong thermal turbulence and temperature inversions, $K=2{\cdot}6\times10^5$ for day flights, and $1{\cdot}6\times10^5$ for night flights.

In addition to counting the total number of grains, Rempe also analysed the grains by species and by size. Usually it was only under the most stable atmospheric conditions at night that any marked sorting out of pollen grains according to size was observed at the different heights; usually the proportions of different sizes were the same at all altitudes, giving convincing evidence of the normally greater importance of turbulence than of gravity on spore distribution. As an example of the sorting out of grains of different species which was occasionally observed, data from one day flight (A6) may be cited. Rempe found: At 2000 m.: *Betula* 73·3%; *Carpinus* 10·0%; *Fagus* 3·3%; and other species 13·4%. At 10–40 m.: *Betula* 29%; *Carpinus* 55·0%; *Fagus* 11·5%; and others 4·5%. Within a single species grains of different sizes were sometimes sorted out as in the night flight (A10) when the mean diameters of *Betula* pollen grains were: 1000 m. 23·0μ; 800 m. 24·5μ; 500 m. 26·7μ; 200 m. 27·5μ; 10–40 m. 27·1μ. Evidently it is only under very low conditions of turbulence that

the differential effect of gravity on pollen grains of different sizes comes into prominence.

From this evidence it seems appropriate to consider the spore or pollen cloud as in suspension in air. At heights of about 1000 m. and upwards the distribution agrees well with that expected from known values of terminal velocity and turbulence. Nearer ground level, however, the suspension tends to become more uniform owing to the intermittent stirring of the lower layers by strong mechanical and diurnal thermal turbulence. As Brunt (1934) points out, the effect of surface turbulence becomes negligible above 1000 m. The result of this stirring, as Horne (1935) has shown by trapping fungus spores over orchards for short periods of time, is that over small areas, micro-organisms are distributed at random in the air. Evidently the observed vertical distribution of air plankton depends on turbulence. Similarly, Kalmus (1936) has demonstrated that turbulence of the medium is also a necessary condition for the existence of plankton in water.

(7) *Spore deposition.* If it is appropriate to regard the spore cloud as a suspension rather than as a shower of particles falling under gravity, the question arises: by what process do they come back to earth?

'When a fluid flows over a solid boundary the portions of the fluid in immediate contact with the boundary are at rest, and there is a thin layer of fluid at the boundary across which there is a rapid increase of velocity, and within which the motion is laminar. The "boundary layer" is a time-mean phenomenon and it is not to be supposed that this layer is always constituted of the same fluid. The turbulence which may prevail at some distance from the boundary will from time to time break through the layer, carrying away portions of the fluid which instantaneously constitute it, but as soon as the individual eddy has removed a portion of the boundary layer normal processes will tend to build it up again' (Brunt, 1934). Above this boundary layer wind velocity tends to increase with height according to some fractional power law.

One of the major problems solved by those land plants that rely on air movement for the dispersal of spores is that of getting the spores away from the surface where they are formed, across the boundary layer of still or non-turbulent flowing air, and into the general circulation of the atmosphere. The adaptation evolved by most Ascomycetes consists in a mechanism for violent discharge of the spores across this layer, and cup fungi by 'puffing' may achieve a ceiling of several inches. Many Basidiomycetes and flowering plants raise the reproductive organ to as great a height as possible, and then drop the spores into the freely moving and turbulent air layers below. Once out of the calm layer of air close to the earth's surface the spores can be freely transported by wind, and the spore cloud is diluted by eddies as it travels. Speed of fall probably plays only a small part in this stage of the dispersal process. But an eddy may at any time bring a spore down into the relatively still ground layer or even to the non-turbulent boundary layer. When a spore is carried down by an eddy into still surface air the effect of gravity would predominate, and here too, if anywhere, the electrostatic forces might be expected to play

a part. In deposition the spore has once again to cross the quiet boundary layer. Here, as Bagnold (1941) points out, dust particles sink into a viscid surface layer of air and are out of the reach of eddies, 'the surface of the ground acts as a sort of dust trap'.

There seem to be no quantitative tests on the deposition of fungus spores, but studies on pollen deposition with special reference to pollen analysis have been made in Germany. Rempe (1937) used two methods for trapping pollen near the ground. Pollen deposition (*Niederschlag*) was measured on horizontal vaselined slides about 1 cm. above the ground. Pollen drift (*Anflug*) in free air was measured by small brass tubes, 14 mm. wide by 45 mm. long, wrapped around the surface with vaselined cellophane, and hung vertically from trees or stakes at the required height (not with cellophane across the ends of horizontal tubes as stated by the Committee on Apparatus in Aerobiology, 1941). In any given position pollen drift was usually much greater than pollen deposition. For instance, at 100 m. in a horizontal direction from a hazel bush the pollen drift at a height of 3 m. was over twenty-five times as great as pollen deposition on the ground. In another series of tests carried out on the level roof of the Göttingen Botanical Institute, values for drift and deposition were obtained over a whole month (Table 2). The fact that deposition was

Table 2. *Pollen 'Drift' and 'Deposition' at Gottingen*

Number of grains per sq.cm. of trap, 1–30 May 1935 (Rempe, 1937).

	Pollen drift 2 m. above roof	Pollen deposition at roof level
Day	23,525	11,200
Night	4,675	6,800
Total of day and night	28,200	18,000
Night as percentage of total	16·6	37·8

smaller by night than by day is interpreted here as due to lower turbulence under night conditions, causing fewer grains to be brought down by eddies to the boundary layer over the horizontal trap. The lower drift values at night do not necessarily indicate a smaller amount of pollen in suspension in the air at night because Rempe's observed drift (spores brought against a sticky surface) will depend on the speed of the wind and its turbulence as well as on the spore content. Actual counts of grains or spores in a measured volume of air would be necessary to establish differences in the number of particles in suspension, but Rempe's aeroplane data, referred to above, indicate that the pollen content by night was only half that during the day. The data in Table 2 show very clearly that by day drift is 50% greater than deposition, while at night, deposition gains in relative importance and is twice as great as drift, apparently on account of decreased turbulence leading to a relative importance of fall under gravity.

By trapping pollen of low-growing plants by both methods Rempe also

showed that there is a zone extending for a few centimetres above ground level which is characterized by predominantly falling pollen.

Rempe also observed that rain had a marked effect in removing pollen from the air, but whether the grains acted as condensation nuclei, or whether they were removed by contact with raindrops falling through the suspension, was left open. In one example, 0·31 grain was trapped per sq.cm. per 4 min. exposure before a thunder shower, during the shower 1·90 grains were deposited, and immediately after the shower only 0·15 grain was deposited, indicating that the pollen content of the air had been halved. During one spell of rainy weather the number of grains trapped decreased steadily to one tenth of its initial value in the course of 2 days. Rain must thus set a limit to the otherwise indefinite process of dispersal by periodically washing the spore load out of the air. For the smaller particles of smoke it has been shown that rain is the principal factor in bringing about deposition, but it must be regarded as incidental for wind-borne pollen and spores, since with many species of plant they are not liberated during rain.

The spore cloud is appropriately regarded as in suspension, and while in process of dispersal in the free air above the ground, spore fall can probably be ignored. In its transport by wind the cloud is acted on by eddies which not only maintain the vertical concentration gradient, but also dilute the travelling spore cloud, which would tend ultimately to a uniform horizontal distribution over the earth's surface if the numbers were not depleted by deposition and by washing out by rain. The factors dominating the actual process of dispersal reduce to two: wind transport and eddy diffusion.

(8) *Eddy diffusion.* The importance of turbulence for dispersing seeds, pollen and spores was first suggested by Schmidt (1918, 1919 and 1925) who derived an equation for the transfer of small particles in the air, an equation which is similar to those for the conduction of heat or for molecular diffusion. A general discussion of this work and the derivation of similar equations for the transfer by eddies of heat, momentum and matter which have been developed by Taylor (1915) and by Richardson (1920) is given by Davies and Sutton (1931), and by Bosanquet and Pearson (1936).

In dealing with the work of Schmidt the monograph (1925) will be used, as the earlier paper (1918) contained an error which was corrected later (1919). Schmidt considered the dispersal of a quantity Q of spores liberated into the air at an instant of time from a fungus growing on a tree. Temporarily it was supposed that the effect of gravity on the spores could be ignored, and it was assumed that each spore remained in a small volume of air and moved with it. By reason of the perpetual mixing of the air by eddying the individual spores become separated from one another. The strength of turbulence determining this is measured by a parameter termed by Schmidt the 'Austausch' value, A. On the basic assumption that the flux of spores across a small layer of air, height δz, is proportional to the gradient of concentration across the layer, and that the increase of spores in the layer is the difference of flux across the two boundaries,

Schmidt considered that changes in the spore content s of the air at a height z above the point of liberation after time t would be given by

$$\frac{ds}{dt} = \frac{A}{\rho}\frac{\partial^2 s}{\partial z^2},$$

where A = the *Austausch* value, ρ = the density of the air. Thus A/ρ replaces the coefficient of thermal conductivity in the heat conduction equation. The integrated form of this is given by Schmidt as

$$S = \frac{Q}{2\sqrt{[(A\pi t/\rho)]}} \exp\left[-\frac{z^2}{4(A/\rho)\,t}\right].$$

Here s gives the distribution of spores in the different layers above the point of origin at time t, and equally in the absence of wind or gravitational pull, it gives the lateral and downward distribution due to turbulent mixing. The spores are normally distributed about the point of liberation with a standard deviation given by $\sigma = \sqrt{(2At/\rho)}$. Schmidt next considered what fraction q/Q of the spores occur at time t above a height z, and extended the treatment to include particles with a speed of fall c in a wind of velocity v cm.sec. The treatment is mainly of historic interest and need not be repeated here. As an example of the method, Schmidt calculated the theoretical horizontal distribution of fruits of the dandelion, *Taraxacum officinale*, with an observed velocity of fall of $c = 10$ cm.sec., in an atmosphere with turbulence given by $A = 20$, and density $\rho = 1{\cdot}293 \times 10^{-3}$, and estimated that 1/100 of the fruits would be carried to a distance of at least 10·1 km. Small (1918), indeed, considered that a wind of only 1·97 miles per hour would carry dandelion fruits to any distance, but this extraordinary result was obtained by neglecting the effect of gravity in the argument and eliminating its effects in the experiment. Defining a 'mean limit of dispersal' as the distance beyond which only 1/100 of all particles travel, Schmidt calculated the following mean dispersal limits from observed terminal velocities of spores and pollen: *Bovista* 460,000 km.; *Polytrichum* 19,000 km.; *Lycopodium* 330 km.; *Pinus silvestris* 40 km.

Similar equations for eddy diffusion had been developed by Taylor (1915) and Richardson (1920), and their application to the scattering of smoke was soon made by Roberts (1923). In these equations some such coefficient as the eddy diffusivity, K, corresponds to Schmidt's A/ρ in similar heat-conduction equations.

Davies and Sutton (1931) pointed out that observed values of A varied from 10^{-2} to 10^{2}, and K from 10^{3} to 10^{11} in c.g.s. units, and it therefore appeared that the 'Fickian' heat-conduction equations do not adequately describe fluids in turbulent motion, and that something more than a scale difference is present. These considerations led Sutton (1932) to put forward a theory of eddy diffusion in which the size of the effective eddy was assumed to increase with the distance travelled by the cloud, instead of remaining constant at all distances as in former theories. Sutton showed that earlier theories assume that the size of eddies effective in diluting the cloud do not vary no matter how widely the particles are dispersed.

On the contrary, he states: 'It is clear to anyone who has watched smoke diffusing that the particles start off at first under the influence of small local eddies, but that at greater distances from the source the scatter is such that the relative motion of the particles is dominated by the larger eddies....In the atmosphere we have a state of affairs in which the agents of diffusion are eddies of all sizes, ranging from the minute convectional whorl to the cyclonic depression. When the particles are closely packed together the large scale eddies will have little or no influence on the rate of diffusion, but when the stage is reached that the particles are widely separated the influence of the smaller sized eddies will become less and less, and the relative motion of the particles, and thus the rate of growth of the cluster, will be determined solely by the larger eddies' (Sutton, 1932, p. 145).

Sutton assumed that the correlation coefficient R_ξ between the motion of a particle at one instant and at a time ξ sec. later varies as an inverse power of the distance travelled by the cluster. (R_ξ behaves like $([u]\xi)^{-n}$, $n>0$). Developing an equation for correlated motion in a fluid, originated by Taylor (1922), Sutton found that with a correlation coefficient of the form proposed the standard deviation of the particles would be different from that given by Schmidt's equation. If σ denotes the standard deviation of the particles from their mean position Sutton found that $\sigma^2=\frac{1}{2}C^2(ut)^{2-n}$, where $0<n<1$, and u = mean speed of the wind. (In what follows $2-n$ is usually denoted by m, and ut replaced by the distance travelled, x.) Here C is a new coefficient of diffusion replacing the K of older theories, and m is a parameter depending on conditions of turbulence.

Table 3. *Observed values of parameters of diffusion equation* (Sutton, 1932)

Author reference	Objects observed	Mean C (cm.)$^{\frac{1}{2}}$	m
Richardson and Proctor, 1926	Balloon competitions on Brighton beach (diffusion over hundreds of kilometres)	0·92	1·75
Richardson, 1920	Smoke from continuous point source (diffusion over tens of metres)	0·6	1·75
Sutton, 1932	Expansion of anti-aircraft shell bursts:		
	At height of 945 m.	0·088	—
	At height of 5400 m.	0·03	—
Defant, 1921 (cited from Sutton, 1932)	Trajectories of European cyclones	1·68	2

The range of values obtained by Sutton for C and m by calculation from various sets of data are shown in Table 3. The relatively constant values of C, compared with the widely divergent values of A and K referred to above are taken as evidence that Sutton's theory gives a closer approximation to the facts of turbulent diffusion than do the earlier theories.

The observed decrease of C with height was expected because at great heights conditions are unfavourable for the formation of eddies. Values for m appear to increase with longer sampling periods, and Sutton suggests that m itself is a function of time. In taking continuous observations over a long period on the density of a cloud he suggests that the random element may become smoothed out so that over a sufficiently long period

$m=2$, and C would become $\sqrt{2}$. These possibilities should be borne in mind when the density formulae described below are applied to some biological data where the sampling period is very long. In a later paper, Sutton (1934) found that in the absence of thermal turbulence the normal value of $m=1{\cdot}75$, but that with convection, m increases. The quantity m is an indicator of the degree of turbulence of the medium and is to a first approximation independent of the mean wind velocity. It is primarily affected only by those factors which tend to damp out or enhance turbulence, such as the vertical temperature gradient and the roughness of the surface. Under conditions of extreme turbulence (temperature decreasing with height) m might approach its upper limit of 2, while with the greatest temperature inversions, in which turbulence is damped out, $m=1{\cdot}24$ as a lower limit.

Expressions for the density in a cloud emitted from various types of source were deduced by Sutton (1932), and for the analogous heat-conduction equations, reference should be made to Carslaw (1921). Bosanquet and Pearson (1936) deduced formulae for smoke and also for dust, in which the effect of gravity in causing precipitation was taken into account. The following conditions considered by Sutton in which the speed of fall of the particles is negligible are believed to be more appropriate to the dispersal of fungus spores:

(*a*) *Instantaneous point source*, such as a puff of Q g. of smoke emitted at an instant of time. Here the origin of the co-ordinates is taken at the centre of the moving puff, and the density of the cloud χ is given by the equation

$$\chi = \frac{Q}{(\pi)^{\frac{3}{2}} C^3 (ut)^{\frac{3}{2}m}} \exp\left\{-\frac{r^2}{C^2(ut)^m}\right\},$$

where r = distance from centre of cloud, u = mean wind velocity.

(*b*) *Continuous point source*, such as a factory chimney emitting Q g. per sec. Here, to obtain an integral that can be handled conveniently, the assumption is made that the spread of smoke laterally and vertically is small compared with its spread down wind. The origin of co-ordinates is taken at the point of emission, and Ox is the direction of the mean wind of u cm.sec. When emission has been in progress long enough for the density distribution to reach a steady state the density is given approximately by

$$\chi = \frac{Q}{\pi C^2 u x^m} \exp\left\{-\frac{y^2+z^2}{C^2 x^m}\right\}.$$

The cross-wind density shows a normal distribution of particles. On the axis of the cloud ($y=z=0$) the concentration is given by the simpler expression

$$\chi = \frac{Q}{\pi C^2 u x^m},$$

and since according to the theory, m cannot exceed 2, the fall off in density on the axis of a point-source cloud cannot be more rapid than the inverse square, no matter how turbulent the motion may be.

(*c*) *Continuous line source at right angles to the mean direction of the wind,* emitting Q g. per sec. per cm., and assuming the line to be of infinite length

$$\chi = \frac{Q}{\sqrt{(\pi)\, Cux^{\frac{1}{2}m}}} \exp\left\{-\frac{z^2}{C^2x^m}\right\}.$$

Values obtained by Sutton suggest that as a rough and ready rule a line behaves as an infinite line for distances of travel up to four times its own length. For points on the xOy plane

$$\chi = \frac{Q}{\sqrt{(\pi)\, Cux^{\frac{1}{2}m}}}.$$

III. Horizontal dispersion of spores

An apparently unique set of data obtained by Stepanov (1935) fortunately makes it possible to test the fit of Sutton's theory of eddy diffusion to spore transport.

Stepanov used artificial sources of spores liberated at a point in the open air, and trapped the spores on glass slides coated with glycerine jelly placed on the ground at various distances from the sources and in various directions relative to the prevailing wind. At the end of the experiment cover-glasses were placed on the slides, and the number of single spores per unit area was counted (spore clusters were disregarded).

In Exp. 1 (28 July 1933), on a lawn near the middle Nevka River, Elagin Island, Leningrad, approximately $1{\cdot}2 \times 10^9$ spores of *Tilletia caries* were disseminated into the air through gauze, at a height of about 0·8–1·2m. above the ground. According to anemometer readings the wind varied from 0·5 to 4·0 m.sec., but sometimes fell off to a complete calm; its direction was also variable. Two glass slides were placed at each locus and the numbers of spores trapped are shown in Table 4.

Table 4. *Results of dispersal of spores of* Tilletia caries, 28 *July* 1933, *Exp.* 1

	Quantity of spores per cover-glass 18 × 18 mm. (average of two)			
Angle to wind	At 5 m. from place of dispersal of spores	At 10 m. from place of dispersal of spores	At 15 m. from place of dispersal of spores	At 20 m. from place of dispersal of spores
−20°.	204	23	4	0
−10°	435	45	19	8
+30°	964	212	207	49
+45°	1198	587	87	142
+55°	659	123	77	15
+65°	341	24	26	7
+75°	365	5	26	53
+85°	20	10	9	14

Exp. 2 (5 September 1933) was conducted in the same place as the previous experiment. A mixture of spores of *Tilletia caries* and *Bovista plumbea* was disseminated through a small sieve at a height of about 1·5 m. Scattering of the spores took 15 min., after which 30–35 min. were allowed

(perhaps unnecessarily) to elapse for the deposition of the spores. The wind varied from 2·3 to 3·0 m.sec. and was sometimes calm. As shown in Table 5, three slides were placed at each trapping locus. Approximately $1{\cdot}8 \times 10^9$ spores of *Tilletia* were used but those of *Bovista* were unfortunately not estimated.

Stepanov's results led him to an empirical law of spore dispersal given as $y = C + a/sx$, where y = the distance at which the spores were trapped,

Table 5. *Dispersal of mixed spores of* Tilletia caries *and* Bovista plumbea, 5 *September* 1933. *Exp.* 2

Quantity of spores per cover-glass 18 × 18 mm. (average of three)

	Tilletia				*Bovista*			
Angle	5 m.	10 m.	20 m.	40 m.	5 m.	10 m.	20 m.	40 m.
−45°	3·0	0·3	0·7	0·0	0·3	0·0	0·0	0·0
−30°	128·0	2·3	0·3	0·0	7·0	0·3	0·0	0·0
−15°	43·3	54·7	4·7	0·3	7·0	4·0	0·0	0·0
0°	206·0	204·0	5·3	8·3	17·0	0·0	1·7	0·0
+15°	623·0	115·3	31·3	1·3	46·0	16·0	0·7	0·0
+30°	877·7	216·7	49·0	7·0	81·3	20·3	6·3	0·0
+45°	911·7	89·7	207·0	9·3	70·0	10·7	7·3	2·7
+60°	245·7	48·0	3·0	2·3	27·7	17·3	0·7	0·0

Table 6. *Calculation of parameters for Sutton's diffusion equation from Stepanov's data* (1935)

Exp. no.			Distance in m. 5	10	15	20	40
1	*Tilletia*	σ	2·25	2·97	5·03	6·64	—
		log σ	0·3522	0·4728	0·7016	0·8222	—
2	*Tilletia*	σ	1·81	3·62	—	4·87	14·79
		log σ	0·2577	0·5587	—	0·6875	1·1699
2	*Bovista*	σ	1·790	3·673	—	5·343	—
		log σ	0·2529	0·5651	—	0·7277	—
	log distance (m.)		0·6990	1·0000	1·1761	1·3010	1·6021

Equation for regression line:

$$a = 0{\cdot}5971 \text{ (S.D. } 0{\cdot}02), \quad b = 0{\cdot}8812 \text{ (S.D. } 0{\cdot}072), \quad Y = 0{\cdot}5971 + 0{\cdot}8812\,(x - \bar{x}).$$

Since $\sigma^2 = \frac{1}{2}C^2x^m$,

$$\log \sigma = \frac{2 \log C - \log 2}{2} + \frac{m}{2} \log x,$$

whence $C = 0{\cdot}637$, $m = 1{\cdot}76$.

x = the number of spores deposited per unit area of trap surface, and C and a are parameters dependent on the conditions of the experiment; s = area of trap surface. The number of spores deposited is thus regarded as varying inversely as the first power of the distance from an origin. It will be shown later that Stepanov's formula, which represents the first experimental approach to the problem, needs modification if it is to describe spore dispersal over a wide range of conditions. First it will be necessary to re-examine Stepanov's data in the light of present knowledge of eddy diffusion.

The observed data enable us to test whether the standard deviation σ, of the spores from their mean position, agrees with Sutton's form: $\sigma^2 = \frac{1}{2}C^2x^m$, or with the older diffusion theories where $\sigma^2 = 4Kt$. The data also enable estimates to be made of the parameters m and C which can be compared with values obtained by previous workers for similar conditions. Examination of the data in Tables 4 and 5 shows that the spores at any one distance do not lie in a smooth normal distribution but are significantly clumped. This is probably due to the short time of the dispersal being insufficient to smooth out the action of a few large-scale eddies. The standard deviations of spores lying at each distance from the source have been calculated in Table 6, where for convenience the deviations from the

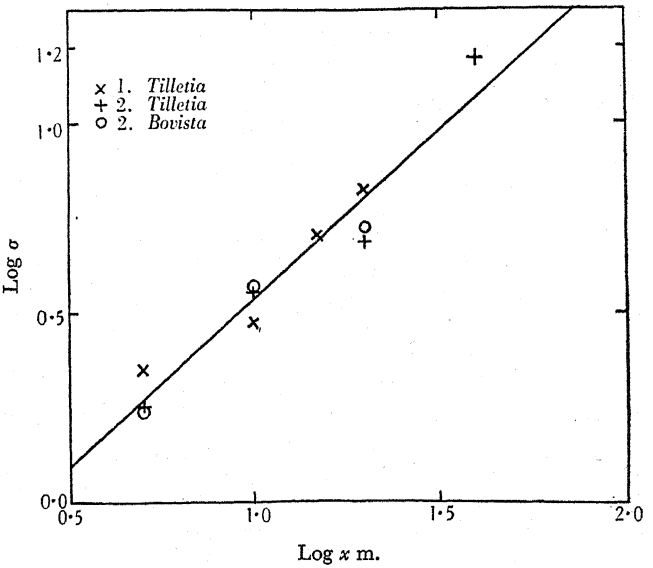

Fig. 2. Stepanov's experiments on liberating spores from a point. Relation between standard deviation of spores deposited at each distance from their mean position, and the distance from source. Also linear regression calculated from data showing agreement with Sutton's eddy diffusion theory where $\sigma^2 = \frac{1}{2}C^2x^m$ ($C = 0{\cdot}64$, $m = 1{\cdot}76$).

mean position at each distance have been measured along the arc with the point source as centre. The standard deviation at each distance has been calculated by the usual formula, $\sigma = \sqrt{[(x-\bar{x})^2/(n-1)]}$. This is not strictly legitimate, since the trapped spores are a systematic instead of a random sample of the population, and should be regarded as estimates of the ordinate of a normal frequency curve. However, the formula clearly gives a useful approximation, which would have been better if traps had extended farther laterally and if data for some of the intermediate radii had not been missing.

Both experiments were carried out in the same place, and with comparable wind velocities, and when values for log σ are plotted against log x, as in Fig. 2, it is found that the points lie reasonably close to a

straight line. The slope of this line is not unity, as it would have been with the older diffusion theories, but corresponds with Sutton's formula for σ, with $C = 0{\cdot}64$ and $m = 1{\cdot}76$. These values agree well with those found by other workers (Table 3), and especially for the exactly comparable data due to Richardson for dispersal of smoke from a point source over distances of tens of metres where $C = 0{\cdot}6$ and $m = 1{\cdot}75$. Sutton's work was apparently unknown to Stepanov when these experiments were carried out, and so the data could not at the time be analysed in terms of eddy diffusion. However, the good agreement between experiment and theory provides strong evidence that spore dispersal in air is mainly controlled by eddy diffusion of the type postulated by Sutton.

Stepanov's cross-wind data have been used to calculate the parameters C and m of the diffusion equation. We can now make independent use of his down-wind data to find out whether the numbers of spores deposited at each distance correspond with the numbers expected from Sutton's density formulae. To do this we shall have to adapt Sutton's formulae to the conditions of the problem, and it will be necessary to find a relation between the concentration of spores in a cloud and the number of spores deposited on a surface over which the cloud travels.

We have to trace the diffusion of a cloud of spores from a point source. It is not possible to assume that a steady state has been set up and that all traps are down wind. For fungus-spore data it is convenient to regard the total quantity of spores liberated, Q, as made up of a large number of small puffs which may or may not take the same direction, but which are all diffused in travel according to the relation $\sigma^2 = \frac{1}{2}C^2x^m$. Accordingly we choose Sutton's formula for an instantaneous point source

$$\chi = \frac{Q}{\pi^{\frac{1}{2}}C^3x^{\frac{3}{2}m}} \exp\left\{-\frac{r^2}{C^2x^m}\right\}.$$

The cloud travels down-wind, and in these experiments its centre is initially at a height z of about 1–1·5 m. above ground level. Except at distances near to the source this will not affect the argument much, and it has in fact been neglected throughout. The surface of the ground is regarded as cutting through the centre of the cloud so that the cloud is not free to expand downwards, and consequently the concentration above the xOy plane will be approximately double what it would have been in the free air. We can represent this by writing $2Q$ in place of Q in the formula. This treatment differs from that of Schmidt, who assumed that any spore which had been calculated to have diffused to the ground level would have been deposited, the ground thus apparently acting as a sieve. In our theory the ground is more appropriately regarded as reflecting the cloud. Any point on the x-axis (at height $z = 0$) will be exposed during the passage of the cloud over it to a succession of volumes of air varying in spore content from an initial zero, up to the maximum of $\chi = \frac{2Q}{\pi^{\frac{1}{2}}C^3x^{\frac{3}{2}m}}$ at the centre of the cloud, and down to zero again. If we imagine a ring around the point source, with radius x, and 1 sq.cm. cross-section, the total number of spores passing through the ring during the passage of

the cloud will be the same as the number in a slice through the cloud in the xOy plane 1 cm. thick. Following Sutton's assumption that the lateral and vertical spread of the cloud is small compared with the distance of travel, and that consequently the density of spores in the cloud remains unchanged during the passage of the cloud across the ring, it can be shown (see Appendix) that the total number of spores, N, flowing through the ring is given by $N = \frac{2Q}{\sqrt{(\pi)}\, Cx^{\frac{1}{2}m}}$. The mean number, n, passing across 1 c.cm. at the distance x is given by $n = \frac{N}{2\pi x}$ or $n = \frac{2Q}{2\pi^{\frac{3}{2}} Cx^{\frac{1}{2}(m+2)}}$. As under any given set of conditions the number of spores deposited from 1 c.c. of air is likely to depend only on the number of spores in that volume of air, we should expect the total deposition, D, on the surface under the ring to be a constant fraction of N, say $D = pN$, and similarly d, the mean deposition per sq.cm. on the ring would be $d = pn$. The number p, which will be referred to as the deposition coefficient, answers the question: if n spores per c.c. flow across 1 sq.cm. of surface, what number will be deposited on the surface? Obviously it is a number of considerable biological importance, for

Table 7. *Number of spores deposited per total trap surface of* 25·92 *sq.cm. at various distances from the point source in Stepanov's experiments*

Exp. no.	Spores of	No. liberated Q_0	Distance in m. 5	10	15	20	40
1	*Tilletia*	$1{\cdot}2 \times 10^9$	4186	1029	455	288	—
2	*Tilletia*	$1{\cdot}8 \times 10^9$	3038·4	731·0	—	301·3	28·5
2	*Bovista*	Unknown	256·3	68·6	—	16·7	2·7

instance, in pollination, as well as in epidemics and their control by protective dusts. Under stream-line conditions p could, of course, be calculated as the resultant of wind speed and the terminal velocity of the particle, as attempted by earlier writers, but little can yet be said about the factors affecting its magnitude under turbulent conditions, and the matter awaits empirical investigation in the field and possibly by wind-tunnel tests where deposition could be studied without progressive dilution of the cloud. Measurements correlating air content and spore deposition are lacking at present. Meanwhile p must be derived from the data empirically.

In Table 7 are shown the sums of the numbers of spores deposited in Stepanov's experiments at all angles to the prevailing wind for each distance from the source. From the values of Q_0 given for experiments with *Tilletia*, and assuming provisionally that there was no diminution of the number of spores in the cloud through deposition, a preliminary calculation shows that the mean number of spores flowing over 1 sq.cm. of a ring on the axial plane of the cloud at a distance of 5 m. would be $n = 6{\cdot}32 \times 10^3$ in Exp. 1, and $9{\cdot}5 \times 10^3$ in Exp. 2. Stepanov's traps at each distance had a total area of 25·92 sq.cm. Actually the traps only sampled a sector of 105° out of a possible 360°, and some few spores were trapped

at most positions on even the most extreme radii, but it is probable that if the traps had been continued round the full circle few more spores would have been caught. Dividing the observed deposition on all the traps at any one distance by 25·92 will give an approximate value for d. With *Tilletia* at 5 m. this operation gives $d=161{\cdot}5$ for Exp. 1, and $d=117{\cdot}2$ for Exp. 2. Since $d=pn$, we can now make a preliminary estimate of the deposition coefficient as $p=0{\cdot}026$ and $0{\cdot}012$ respectively in the two experiments.

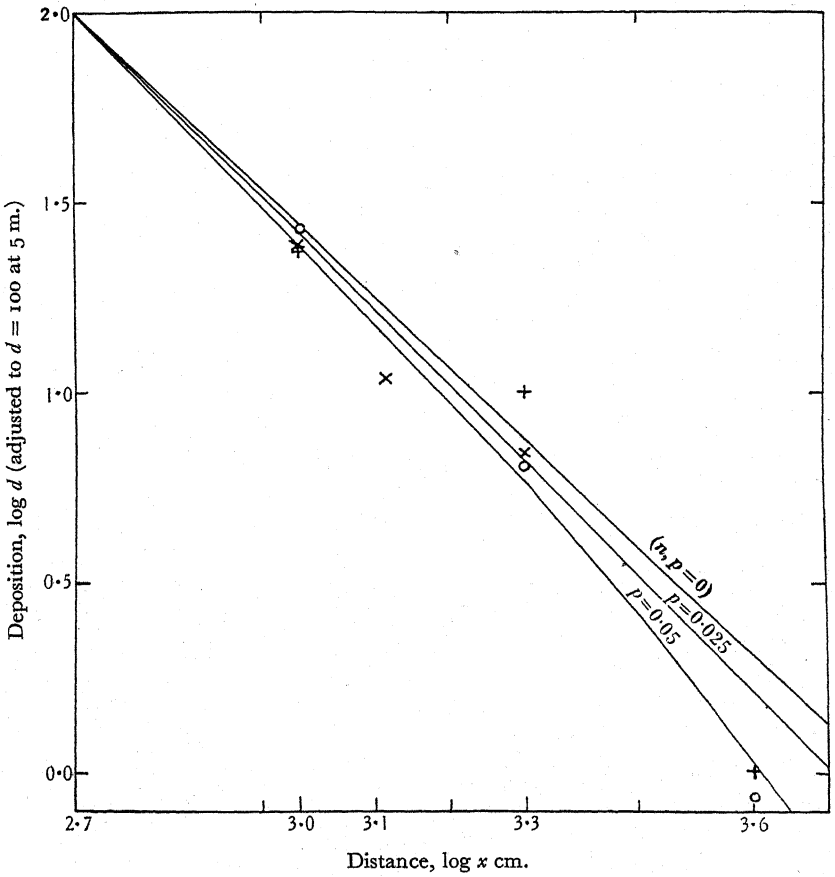

Fig. 3. Stepanov's experiments on liberating spores from a point. Relative numbers of spores deposited, and distance from source (all directions pooled, and each series adjusted to density of 100 at 5 m.). Also curves showing d expected with two trial values of the deposition coefficient, p and n for $p=0$.

Sutton's formulae were developed for calculating the densities in a cloud of particles whose deposition was negligible, the number of particles in the cloud, Q, remaining constant throughout the diffusion. In our problem, although the effect of gravity on dispersal has been shown to be small and has been neglected, the quantity of spores is steadily diminishing

owing to a relatively large deposition from that part of the cloud in contact with the ground, so that Q_x, the total quantity remaining in the cloud when its centre has moved a distance x, is less than the original Q_0. It can be shown (see Appendix) that Q will decrease exponentially with increasing distance according to the equation

$$Q_x = Q_0 \exp\left[-\frac{2px^{(1-\frac{1}{2}m)}}{\sqrt{(\pi)}\,C(1-\frac{1}{2}m)}\right].$$

Values of Q_x and d for two values of the parameter m have been computed (see Appendix, Table 21).

It is now possible to test the theory against Stepanov's data. In each of his experiments the original number of spores liberated differed, so the data must first be put on a comparable basis by equating the mean deposition, d, at 5 m. to 100%, and then expressing the deposition observed at greater distances by relative percentages. In Fig. 3 the logarithms of the observed relative depositions are plotted against the logarithms of the distances in centimetres. The expected depositions when $p=0{\cdot}05$, $0{\cdot}025$ and zero respectively are also plotted. As p decreases the slope of the line of expected densities also decreases, and approaches the limiting value of $\frac{1}{2}(m+2)$. It will be seen from Fig. 3 that the line calculated for $p=0{\cdot}05$ approaches most closely to the observed values. An increase over the provisional value for p obtained in the preliminary calculation, when Q was assumed to remain undiminished by deposition, was to be expected, as Table 21 shows that about a quarter of the spores liberated would probably have been deposited by the time the cloud reached a distance of 5 m. A deposition coefficient of $p=0{\cdot}05$ means that in travelling across 1 sq.cm. of surface the entire cloud would deposit a quantity of spores approximately equivalent to the number contained in a slice half a millimetre thick through the axial plane of the cloud xOy. Mean values for wind velocity during the experiments were not given, but from the data would probably not be less than 1 m.sec. At this speed of travel 0·5 mm. of cloud would be cleared in about 1/100 sec., the time taken to travel a distance of 1 cm. This represents about five times the thickness of the cloud that would be expected from the known terminal velocity of fall of spores of *Tilletia* in still air, and may indicate that particles are deposited more rapidly from turbulent than from still air.

IV. Plant-disease gradients

Stepanov's experiments give evidence that Sutton's eddy diffusion theory is applicable to the dispersal and deposition of dry fungus spores in air under average day conditions from a point source, provided that the diminution of the number of particles in the cloud through deposition is taken into account. No other available experiment allows the various parameters for the field to be estimated, but in plant disease literature there are records giving quantitative data on the numbers of spores, lesions, infected individuals, etc., observed at different distances from the presumed source of the disease. Before the deposition formulae can be

applied to plant-disease gradients there are several difficulties to be considered.

Meteorologists have been interested principally in diffusion during short periods of time of the order of 10–100 min. during which the wind can usually be considered to have a mean direction and velocity. Biological data often refer to sampling periods of days or even months, during which the wind undergoes major changes in direction and velocity. Changes in wind velocity, without changes in the direction, are, however, on Sutton's theory relatively unimportant. Since $\sigma^2 = \frac{1}{2}C^2x^m$, the density of the cloud depends on the distance travelled by the cloud and not on the time taken to travel that distance. Changes in wind direction, however, introduce a factor which cannot be dismissed so easily. Two special cases can be considered here. When records are available in all directions around a symmetrical source the mean deposition per sq.cm. irrespective of direction is given by d. If dispersal has taken place while the wind came from a uniform direction, as may happen when conditions for the spread of a disease are favourable for periods of only an hour or two as with potato Late Blight, then if observations are made only at points down-wind the relative deposition will be given by d_w (Table 21). This down-wind deposition gradient is very similar to d, but slightly flatter under normal conditions and steeper under low turbulence conditions.

The deposition equation gives the number of particles expected when the parameters Q, m, C and p are known. Measurements of these have not been made so far in connexion with any plant disease, and it will be necessary to assume values for average conditions of the atmosphere found by other methods. Also, since the strength of the source of infection varies greatly from field to field, the equation will give only the relative numbers of spores deposited at each position. This number is probably always many times greater than the number of spores which are successful in infecting plants and causing lesions, and must vary greatly under different conditions. The proportion should be uniform, however, over the imaginary uniform field which we postulate. All these factors influence the height of the curve above the x-axis but do not affect its shape.

When disease data are given as the number or percentage of plants attacked irrespective of whether the plant has one or many lesions, aphid punctures, etc., the percentage will have to be suitably transformed before the formula for deposition can be applied. The method used was worked out by Thompson (1924) who gave a table from which the necessary correction can be made. The conversion, which becomes necessary only when the proportion of infected individuals is above about 20 %, will be referred to as Thompson's transformation. It depends on the fact that if in a group of say 100 individuals there is only one lesion, then obviously only one individual, or 1 %, can be infected. A second lesion occurring at random on the same group of 100 individuals will have one chance in 100 of infecting the individual that already has one lesion, and so on. It can be shown that to infect 50 % of the individuals in a large population it would take about seventy randomly distributed lesions per 100 individuals. Conversely, the table can be used to detect non-random distribution, due

to such factors as leaves in different positions on a plant not being equally suitable environments.

Stakman (1942) rightly stresses the need for experiments with 'marked' spores or pollen grains, easily identifiable and of known origin, in order to facilitate the development of the principles of aerobiology. Many spores are of almost ubiquitous occurrence in the air, and it is impossible to know from whence they come. This fact raises the question in interpreting field data of whether a significant proportion of the spores trapped, or lesions counted, represent contaminations from sources other than the one under consideration. The effect of any general contamination will consist of a uniform addition to the observed deposition at all distances from the particular source under investigation. The effect of this addition will be to flatten the gradient so that, plotted on a log-log scale, the line would ultimately become parallel to the x-axis. Most of the data from field observations which are quoted below do not show such an effect, but one or two examples are given.

The basidiospores of rusts and the sporangia of downy mildews are known to be relatively short-lived when dry, but there seems to be no evidence that death of spores in transit would decrease the number of successful infections over the distances of tens or hundreds of metres discussed in this paper. In a wind as slow as 0·5 m.sec. the sporidia of *Cronartium ribicola* would take only about 10 min. to travel the 900 ft. regarded as the normal limit for infection of pines by this fungus. From data on the viability of its sporidia given by Spaulding and Rathbun-Gravatt (1926) the absence of disease on plants at 900 ft. seems more likely to be due to the great dilution of the cloud, making infection very improbable.

Field observations are complicated by the fact that sometimes records were not made until after lesions derived from the original source had themselves multiplied and acted as new sources for the spread of the disease. As far as possible, such complications have been avoided in the examples chosen below. The effect would be to flatten the observed gradient, because we should have to deal not only with the original source, but also superimposed on this the effects of a series of contaminations varying according to the distance from the original source. Such cases cannot be dealt with systematically at present.

The deposition formula can now be compared with data from published papers on plant-disease gradients, where the numbers of spores, lesions or diseased plants have been counted at various distances from a source of disease. Many published observations have had to be excluded because one or more essential measurements are not clearly given. References to disease gradients are frequent but quantitative studies are very rare, though no doubt many sets of data have been overlooked in the present survey. It should be pointed out that observations have been gathered by the workers concerned as part of routine studies and without any reference to a possible general study of gradients. This adds interest to the following collection of data, most of which agree reasonably well with the deposition expected from the eddy diffusion theory, but one or two of which show marked divergences.

In the following examples the data are plotted in Figs. 4 and 5 on a log-log scale. On the y-axis is plotted $\log_{10}$ observed number of spores or lesions, and on the x-axis is plotted $\log_{10}$ of distance from the source in centimetres. For comparison two theoretical lines are also plotted for the appropriate formula, one is for ordinary turbulent conditions with $m = 1{\cdot}75$, and one for very low turbulence with $m = 1{\cdot}24$. Q_0 for these theoretical lines is chosen as 10^{10} in Fig. 4, which is a number of spores of the order that might be encountered in the field. (Christensen (1942) found that an acre of wheat moderately affected with *Puccinia graminis* would produce at least 10^{11} spores, and Buller (1922) estimated that one

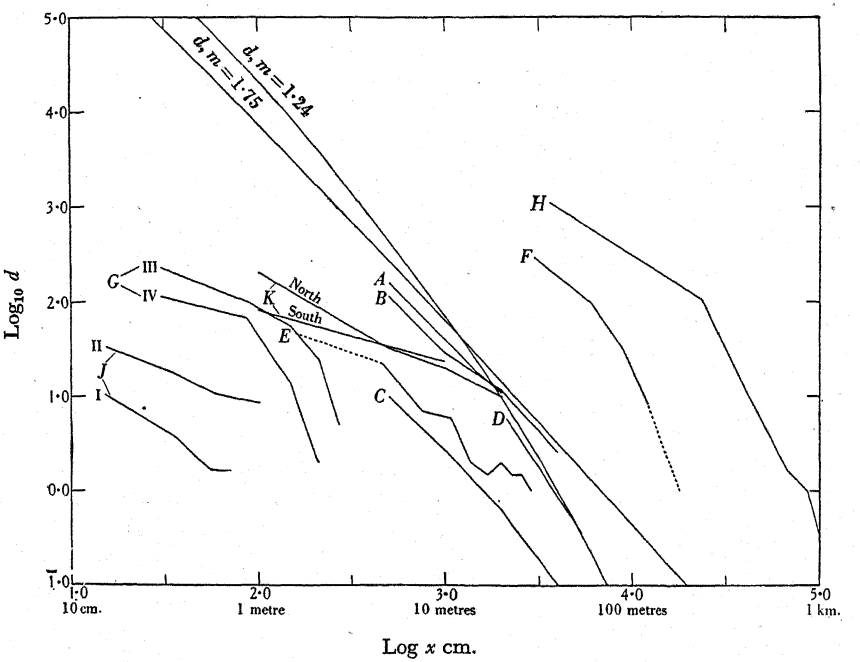

Fig. 4. Observed plant disease gradients from a point source, plotted on double log scale. Also lines showing expected values for normal and low turbulence conditions ($m = 1{\cdot}75$ and $1{\cdot}24$ respectively) calculated for $Q_0 = 10^{10}$ spores. *A* and *B*, *Tilletia caries* spores, Stepanov; *C*, *Bovista* spores Stepanov; *D*, *Puccinia coronifera* spots on oats, Grushevoi; *E*, *Cronartium ribicola* cankers on pine, Buchanan and Kimmey; *F*, *Phytophthora infestans*, Bonde and Schultz, on potato; *G*, *P. infestans* on potato, Limasset; *H*, *Peronospora destructor* on onions, Newhall; *J*, *Botrytis tulipae* on tulip, Wallace; *K*, *Cercosporella herpotrichoides* on wheat, Oort.

puff-ball, *Calvatia gigantea*, produced 7×10^{12} spores.) To atone for our previous arbitrary treatment of the deposition coefficient, due to lack of experimental data, the value for p found in Stepanov's experiments has been adhered to throughout and so has the value for C. Subsequent work should throw more light on the variability of these parameters.

When field data for passively air-borne spores are plotted on the same graph the slope of the line at any distance should not normally be less

than the slope of the theoretical line for $m = 1{\cdot}75$ at the same distance. With organisms which liberate their spores and initiate infection under very humid conditions of fog, or in the early morning hours when diurnal turbulence is at a minimum, such as potato blight (*Phytophthora infestans*), the observed gradient might be expected to approach the theoretical curve for low turbulence where $m = 1{\cdot}24$.

(1) *Gradients from a point source.* Data on the dispersal of spores of *Tilletia caries* and *Bovista plumbea* obtained by Stepanov (1935) have already been described in detail. All three series (Fig. 4 *A*, *B* and *C*) agree reasonably well with the slope of the theoretical line for normal turbulence. In *Tilletia caries* the lines are lower than the theoretical line, which was calculated for $Q_0 = 10^{10}$, by an amount proportional to the strength of the source (Exp. 1, $Q_0 = 0{\cdot}12 \times 10^{10}$ and Exp. 2, $Q_0 = 0{\cdot}18 \times 10^{10}$).

Table 8. *Crown rust* (Puccinia coronata) *on oats near a* Rhamnus *bush*

Mean number of lesions per plant (Grushevoi, 1929, cited from Stepanov, 1935)

Distance from buckthorn bush	3 July Mean no. lesions per plant	13 July Mean no. lesions per plant
'Directly neighbouring'	10·38	24·38
10 sazhen (70 ft.)	5·70	19·09
25 sazhen (175 ft.)	0·35	6·85

A gradient of infection of oats with crown rust (*Puccinia coronata*) in proportion to the distance from an infected bush of *Rhamnus*, the alternate host, was described by Grushevoi (cited from Stepanov, 1935: see Table 8). Two points only are available for plotting, as the nearest of the three samples was merely stated to be 'directly neighbouring' the buckthorn bush. Counts were made on two dates. The count made on 3 July when the plants were in the flowering stage agrees well with the gradient expected for average conditions (Fig. 4 *D*). Ten days later another set of counts was made and a flatter gradient observed, presumably owing to the secondary spread of the disease.

Blister rust (*Cronartium ribicola*) is perennial in the stems of five-needled pines. On this host it produces aecidiospores which are capable of infecting only the leaves of the currant (*Ribes* sp.). On the currant are produced uredospores capable of infecting other currants, and also teleutospores which on germination produce the sporidia (basidiospores) which are able to infect only pine needles. Spaulding (1920) noted a regularity about the gradient of infection on *Pinus strobus* observed by Prof. Pennington in the Adirondacks: 'In general, it may be said that under given conditions the number of infections in pine varies directly with the amount of *Ribes* leaf surface, and inversely as the square of the distance from *Ribes*.' Blister rust gradients were studied experimentally on *Pinus monticola* in British Columbia by Buchanan and Kimmey (1938). Blocks of uninfected pine, 22·5 acres in area, were cleared of all currant bushes except for a group at the centre of the plots. These currants were inoculated to serve as a source of infection. *Ribes* was also removed from various zones around the plots. The infections on the pines resulting from spores produced by

diseased currants were sometimes distributed evenly in all directions, but when the plots were on slopes the lesions tended to be more numerous down the slopes. Some of Buchanan and Kimmey's tables show marked gradients, but as data are expressed in units of 'trees' of very diverse size they are not suitable for our purpose. From one of their graphs, however, can be read the pooled results of all their experiments in terms of cankers per million leaves, and the data are given in Table 9. When plotted (Fig. 4 *E*) the gradient shows a general agreement with the expected distribution, but also illustrates one or two points of discrepancy. The first point plotted on the graph is entered at 5 ft., which is the mean of the first zone measured from the plot centre, but as this central zone differs from all other zones in being partly occupied by the *Ribes* source instead

Table 9. *Average number of cankers of* Cronartium ribicola *per million* Pinus *leaves exposed in* 10 *ft. zones out from central* Ribes. (Buchanan and Kimmey, 1938. Data for all plots combined)

Distance from plot centre (ft.)	No. cankers per million leaves
0–10	46·5
10–20	22·5
20–30	7·0
30–40	6·0
40–50	2·0
50–60	1·5
60–70	2·0
70–80	1·5
80–90	1·5
90–100	1·0
100–110	2·0

of pine trees it is not surprising that the number of cankers recorded should be lower than the theoretical value. The second point of interest is that beyond about 60 ft. the number of lesions becomes practically constant, suggesting that in some of the plots which contribute to the data a contamination on a low level may have been coming in from outside. A similar effect from a contamination was observed by Brunt (1932) for smoke pollution around the City of Norwich.

Gradients for a disease with a similar life cycle were given by Schneiderhahn (1926) in studies on cedar rust of apples (*Gymnosporangium juniperi-virginianae*), but unfortunately they cannot be used because information on the magnitude of the source is lacking.

Gradients of two sets of data of infection due to potato Blight (*Phytophthora infestans*) are available, but neither is well suited to our purpose. One of great interest is that of Bonde and Schultz (1943), who traced blight developing in potato fields in Maine to its origin in waste potato dumps of the previous year's crop. One of their tables gives the number of lesions at specified distances from one such dump which we can treat as approximating to a point source. The counts were presumably taken down wind in the direction of greatest spread, though this is not definitely stated. Their table gives both the percentage of plants infected and the number of lesions per 100 plants. Applying Thompson's trans-

formation to the percentage figures we find that the observed numbers of lesions are of the same order as would be expected if their distribution at each distance were random, and so it can be concluded that the lesions observed do in fact represent infections coming from the primary focus and that the position is not complicated by local spread from a second generation of spores (Table 10). The first point of the curve (Fig. 4 *F*) is unexpectedly low, perhaps owing to the difficulty of finding as many as 100 plants at exactly 100 ft. from a point source, but the remainder of the gradient is compatible with dispersal under low turbulence conditions.

Table 10. Phytophthora infestans. *Late blight in potato field near infected refuse pile in Maine* (Bonde and Schultz, 1943)

Distance from refuse pile ft.	Plants infected % (observed)	Lesions per 100 plants (observed)	Lesion no. expected from Thompson's transformation
100	98	293	395
200	55	98	80
300	21	31	24
400	6	9	—
500	0	0	—
600	1	2	—

Table 11. *Potato plants infected with* Phytophthora infestans *near primary foci on* 31 *July* (Limasset, 1939)

Mean distance from edge of infector group cm.	No. infected total plants	Percentage infected *y*	Probable lesion no.
	Focus III		
30	19/28	68	114
90	15/32	46·8	63
150	6/44	13·6	14
210	1/50	2·0	2
	Focus IV		
30	25/28	89	221
90	20/32	62·5	97
150	19/44	43	53
210	11/50	22	25
270	3/60	5	5

The fluctuation at 400–600 ft. illustrates the importance of increasing the size of the sample when the number of lesions is low.

Another set of data on *Phytophthora infestans* comes from Limasset (1939), who investigated the role of diseased potato seed tubers in forming primary foci when planted in a crop. Compact groups each of twenty infected tubers of the variety Early Rose were planted in a plot of the variety Saucisse to act as a source of the disease. On 28 July, after a rainy period, blight appeared on some of the shoots emerging from infected tubers, but at that time the disease was not seen elsewhere in the district. The disease spread to surrounding plants, and from the data given and a plan of the field, scale diagrams have been made from which Table 11 was prepared. The shape of the source in these experiments was approximately

a circle of 200 cm. radius, and the effect of the disk source has obviously been to flatten the gradient (Fig. 4 *G*), especially at points near to the source; so to regard this as a point source can only be a very rough approximation. However, the agreement in general trend of the gradients on the two plots is worth noting.

Downy mildew on onions (*Peronospora destructor*) was studied by Newhall (1938) in Wayne County, N.Y. The source consisted of a tenth of an acre of onion bulbs grown for seed in the north-west corner of a valley (a similar source lay a quarter of a mile back). Counts were made of the number of lesions on different onion fields, grown for the bulbs, at various distances from the source down the valley. It will be appropriate to treat the source as a point in relation to most of the fields, though the nearest readings might be expected to show the effect of the area of the source (see following section). The dispersal is not over a uniform field but refers to a number of fields in which conditions may be expected to differ considerably. The data given in Table 12, when plotted (Fig. 4 *H*), give a gradient suggesting dispersal under low turbulence conditions.

Table 12. *Dispersal of onion downy mildew lesions from a point source* (Newhall, 1938)

Distance from source (ft.)	No. of lesions per 100 ft. row
120	1135
780	107
1750	4
2200	1·7
2800	1·0
3300	0·33

In the two cases to be described next the data are not compatible with the scattering expected by eddy diffusion. They are gradients of infection with *Botrytis Tulipae* and *Cercosporella herpotrichoides*.

Wallace (1934) studied the distribution of secondary leaf spots on beds of tulips (William Copland) growing out of doors in Lincolnshire, and gave diagrams showing the number of spots on plants situated near a tulip shoot bearing spores of *Botrytis Tulipae*. In a letter, Mr E. R. Wallace states that the bulbs were planted about 5 in. apart in rows 7 in. apart, with a path of 18 in. between the beds, and from a scale diagram Table 13 was prepared. On plotting the log number of lesions per plant against the log distance in centimetres, we obtain gradients (Fig. 4 *J*) quite incompatible with the eddy diffusion theory. The minimum possible slope (even under very low turbulence and with no diminution in Q_0 through deposition) would be $\frac{1}{2}(m+2) = 1{\cdot}62$, whereas the first bed has a gradient of about 1·3 and the second as low as 1·0. Beaumont, Dillon Weston and Wallace (1936) state that *Botrytis Tulipae* is dispersed in the field by two methods, wind and splashing in rain drops. It is suggested in explanation of the anomalous gradients that they are examples of splash dispersal and not of wind transfer. Examination of the weather records kept at the Kirton Agricultural Institute where the crop was growing supports this

explanation. Disease records were made during a dry period, but in each case over a tenth of an inch of rain fell 5 days prior to making the counts. The rainfall records were: 20–30 March, nil; 31 March, 0·12 in.; 1 April, nil; 2 April, 0·1 in.; 3–8 April, nil; 9 April 0·13 in.; 10–17 April, nil. (These records are published by kind permission of the Principal of the Kirton Agricultural Institute.) Unfortunately, nothing is known about

Table 13. *Distribution of spots on tulip plants near to shoot-bearing conidia of* Botrytis Tulipae (Wallace, 1934)

Mean distance from focus (cm.)	Mean no. lesions per plant
First bed (focus removed and spots counted 4 April 1933)	
15·2	10·5
35·8	3·72
54·4	1·67
Second bed (spots counted 13 April 1933)	
15·3	33·4
34·6	17·9
58·0	10·7
79·8	9·48
102·0	8·70

Table 14. *Influence of the distance from the source of infection on the occurrence of* Cercosporella herpotrichoides

Distance from source of infection m.	Percentage culms with eyespots	Probable no. of lesions (Thompson's transformation)
	To north	
1	87	205
5	28	35
10	18	20
20	10	10
35	8	8
50	11	11
60	11	11
70	1	1
	To south	
1	56	82
10	26	30

the type of gradient given by splash-dispersed spores, but it is worth noting that the gradients given by this *Botrytis* are similar to that found in the undoubted case of splash dispersal that follows.

The spread of Eye-spot of wheat (*Cercosporella herpotrichoides*) was studied by Oort (1936). In the middle of a field of autumn-sown wheat, where the disease had not previously appeared, twenty plants badly attacked by the fungus were transplanted to a small patch in December 1934. In the following July samples of about 200 culms each were collected at different distances from the diseased patch and inspected for Eye-spot with results given in Table 14. When plotted (Fig. 4 *K*) the gradient obtained is totally incompatible with the eddy-diffusion theory (still more so after

applying Thompson's transformation). There is a suggestion in Oort's data that in addition to the lesions derived from the source, there may have been some infections due to a general contamination from the same or neighbouring fields. Over the experimental field itself, Oort recorded the presence of small secondary infections high up the culm, and stated that only within about 10 m. of the artificial source were infections to be found at the foot of the culm. The figures show no obvious decrease after 10 m., but only what are probably fluctuations due to sampling, so the possibility is that there is a contamination averaging about 8 % not due to the source. When plotted the gradient, which mainly represents the effect of the artificial source, is flatter than demanded by the eddy-diffusion theory. This is of interest when taken in connexion with the findings of Sprague and Fellows (1934), who state that Eye-spot increases suddenly in the north-western United States after heavy rains in spring; that spores are carried by splashing water; but that there is some evidence that spores are sometimes air-borne. So that, although Mason (1937) puts *Cercosporella* among the dry-spored fungi, the evidence is that this species is normally dispersed by water, and it is therefore not surprising that the eddy diffusion formulae should not apply. Alternatively, it is possible that the original gradient has been flattened by local secondary infections.

(2) *Gradients from a line source.* The deposition formula so far used is appropriate for particles scattered from a point source. With many sets of data, however, the source is significantly long, or both long and wide. It would then be appropriate to use a formula derived by integrating the effect of all sources over the line or area from which the particles are being dispersed. Such an integration cannot conveniently be dealt with. When more data are available on the various parameters of the diffusion equation it will probably be worth while to carry out mechanical integration for sources and samples of standard dimensions and for standard sets of conditions, but for the present only an approximation can be attempted. This can be done by integrating the effect of an infinite line source Oy, liberating Q_0 spores per cm. while the wind is blowing in the direction Ox. The deposition per sq.cm. at a distance x cm. down wind from an infinite line source is then given approximately by

$$d_{lw} = \frac{p\,2Q_x}{\sqrt{(\pi)}\,Cx^{\frac{1}{2}m}} \quad \text{(see Appendix, Table 21)}.$$

Sutton observed that a finite line source appears to behave as an infinite line source for distances up to four times its length (this is because the lateral effect of parts of the source which are not up-wind is very small), so this approximation would be appropriate where the source is a crop row, a hedge, or a weed strip on a headland, etc. At any point the major contribution to the deposition would be expected to be that carried by winds which were directed along the x-axis. Little more can be said in justification of the approximation except that in practice the gradients from line sources described in the next few examples are flatter than would

be expected from a point source, and approach the curve expected from this formula.

At points very close to the source its width will have to be taken into account. It will be evident that deposition from a strip will fall off less rapidly than from a line source, just as deposition from a line source falls off less rapidly than from a point. Further, while a 10-acre field ought to be treated as a strip source for points in adjoining fields, for fields a few miles away it would be appropriate to choose the formula for a point source.

The first gradient from a line source to be described is unusually steep; it refers to trapping of aecidiospores of *Puccinia graminis*. Lambert (1929) quotes some observations of Christensen at Northfield, Minnesota, where a barberry hedge, consisting of 175 heavily infected bushes, most of them 6 ft. or more high, was found on a deserted farmstead. The total production of aecidiospores from the hedge was conservatively estimated at over 1000×10^{10}. Vaselined glass slides were exposed vertically 3 ft. above the ground on 24–25 May 1932, for a period of 20 hr. during a period when spores were being liberated at a tremendous rate. The numbers of spores trapped at different distances from the hedge are shown in Table 15.

Table 15. *Numbers of aecidiospores of* Puccinia graminis *trapped near a barberry hedge* (Lambert, 1929)

Distance from barberry hedge ft.	No. of spores trapped per slide 3 × 1 in.
3	160,000
6	33,000
23	210

Other slides were exposed on other dates and for different lengths of time, and some spores were trapped up to a mile from the bushes. Plotted on a log-log scale this decrease in deposition represents an exceptionally steep gradient (Fig. 5 *A*), not incompatible with the eddy-diffusion theory, but indicating either a higher value for the deposition coefficient, p, than that assumed hitherto, or that the traps were not down wind. Aecidiospores of *P. graminis* are discharged singly or in clumps to a distance of 4–5 mm. from the cup, and discharge takes place only in an approximately saturated atmosphere. Buller (1924), who studied the phenomenon, also reported the occasional discharge of what he called 'aecidiospore-bombs', or clumps containing 60–150 individual spores. The discharge of spore aggregates as well as single spores might explain an unusually high value for p.

The dispersal of aecidiospores of *P. graminis* from a barberry hedge was also studied by Johnson and Dickson (1919), but instead of trapping spores they counted infected stems of *Agropyron repens*. From their diagram the hedge seems to have been about 100 yd. long running north and south, and observations were made at distances up to 425 yd. from the hedge in a north-easterly direction. The line-source formula should therefore be reasonably appropriate for the data given (Table 16). The samples apparently consisted of 200 plants at each locus, and larger samples would

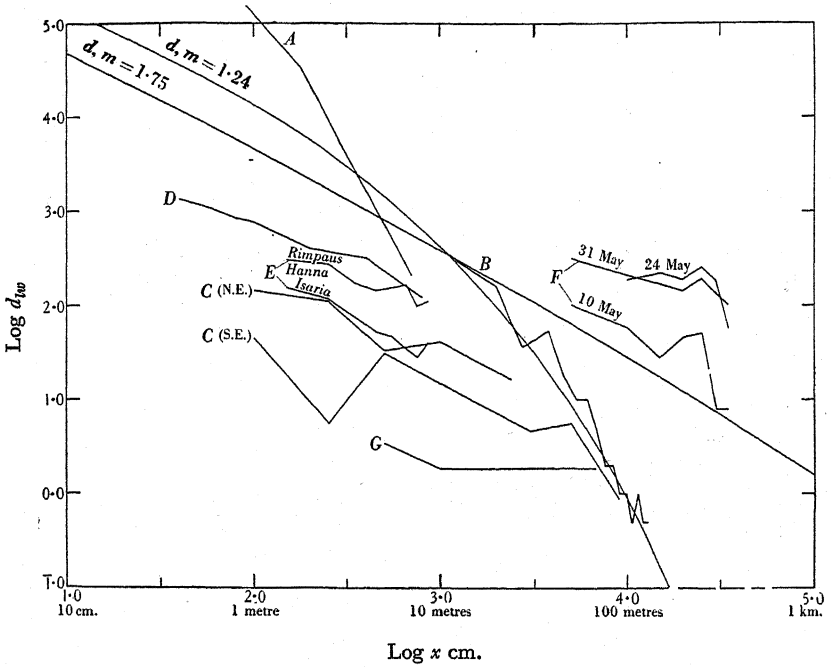

Fig. 5. Observed plant disease gradients from line and strip sources, plotted on double log scale. Also lines showing expected values for ordinary and low turbulence conditions, calculated for $Q_0 = 10^7$ spores. *A*, *Puccinia graminis* aecidiospores, Lambert; *B*, *P. graminis* spots on grass, Johnson and Dickson; *C* and *D*, *Tilletia caries* on wheat, Oort; *E* and *F*, *Erysiphe graminis* on barley, Pape and Rademacher; *G*, *Puccinia coronifera* on oats, Gassner.

Table 16. *Spread of* Puccinia graminis *to grass from rusted barberry hedge* (Johnson & Dickson, 1919)

Distance from barberry hedge ft.	Percentage grass stems infected	Probable no. of lesions by Thompson's transformation
15	100	—
40	95	300
65	80	165
90	30	36
125	41	54
150	15	17
175	10	—
200	10	—
225	5	—
250	2	—
275	2	—
300	1	—
325	1	—
350	0·5	—
375	1	—
400	0·5	—
425	0·5	—

have been required to obtain more accurate estimates of the percentage infection at the farthest distances. At 15 ft. 100% of the plants were infected, and so no estimate can be made of the number of spores successful in establishing lesions at that distance, but estimates can be made at distances of 40 ft. and upwards by applying Thompson's transformation. When plotted (Fig. 5 *B*) a steep gradient is obtained which is compatible with dispersal from a line source under low turbulence conditions.

Loose smut of wheat (*Ustilago Tritici*) was studied by Oort (1940) in connexion with the Dutch scheme for issuing health certificates for seed wheat, to find how far the health of a crop was affected by disease on neighbouring fields. In one experiment at Wageningen a rectangular strip of wheat which had been artificially heavily contaminated with loose smut

Table 17. *Spread of loose smut of wheat* (Ustilago tritici) *at Wageningen* (Oort, 1940)

Distance from strip source m.	No. smutted heads in sample of seed*
Direction north-east	
1	145
2·5	113
5	33
10	40·4
24	16·6
Direction south-east	
1	45
2·5	5·6
5	31·4
10	14·8
25	4·6
50	5·5
90	0·9

* Mean number of smutted heads per pair of plots of area 200 sq.m. from seed sampled at each distance.

was bounded on the north-east and south-east sides with smut-free wheat. At harvest time, samples were taken at different distances from the diseased rectangle in both north-east and south-east directions, and the seed was sown in the following season and the numbers of loose smut *heads* counted. In Table 17 is given the number of smutted heads for a sample area of 100 sq.m. This area would contain about 20,000 heads, and as the highest number of smutted heads recorded was only eighty-five, or less than 1%, it is clear that Thompson's transformation need not be applied. The question if smut-infected plants tiller as freely as healthy ones is also irrelevant since the numbers at the different distances are only relative.

Another experiment was carried out at Sprundel on a field situated at a great distance from other wheat fields. The field was sown with wheat which had been carefully treated with hot water, except for a small plot 10 m.sq. sown with seed of the same variety heavily infected with loose smut to serve as a source of infection for the rest of the field. This square had 1350 smutted heads in the summer of 1937. In August, samples were

harvested in different directions and distances from the contaminated square. These were planted in the following season. The numbers of smutted heads per 100 m. of plot grown from these samples is shown in Table 18 for distances from 0 to 8 m. in the north, south, east and west directions. (Data for other directions and distances are incomplete.) Both

Table 18. *Spread of loose smut of wheat* (Ustilago tritici) *at Sprundel* (Oort, 1940)

Distance from square source m.	Total smutted heads in sample plots of 400 sq.m. in all directions from source
0·4	1362
0·6	1082
0·8	857
1·0	769·9
1·2	507·9
2·0	403·5
4·0	319·4
8·0	121

Table 19. *Spread of* Erysiphe graminis *from winter to spring barley at Kitzeberg, Schleswig-Holstein* (Pape & Rademacher, 1934)

Distance from strip source (mean) m.	Percentage plants affected (mean of S.W., N.E., S.E. and N.W. directions)	Probable lesion no. (Thompson's transformation)
Variety *Isaria* (mean of four directions)		
1·5	79	156
2·5	69·5	119
3·5	52·5	75
4·5	41	53
5·5	36·2	47
6·5	30	36
7·5	26·2	30
8·5	32·2	40
Variety *Rimpaus Hanna* (mean of two directions)		
1·5	95·5	310
2·5	93·5	275
3·5	82·5	177
4·5	76·5	145
5·5	79·0	156
6·5	80·0	161
7·5	60·5	93
8·5	67·5	112

these experiments of Oort's give similar gradients (Fig. 5 *C*, *D*) which are compatible with diffusion down wind from a strip source under normal atmospheric conditions.

Gradients from strip sources were observed by Pape and Rademacher (1934) in studies on the spread of powdery mildew (*Erysiphe graminis*) from winter barley to adjacent spring-sown barley in Schleswig-Holstein. One at Kitzeberg, for which data are most complete, consisted of a plot of winter barley 7 × 13 m., surrounded by spring-sown barley. On the day after which mildew spots first appeared on the surrounding spring-sown

barley, the percentage of plants infected was counted at a series of distances from the edge of the plot in several directions. Results for two varieties given in Table 19 show a gradient (Fig. 5 *E*) flatter than would be expected from a point source, but compatible with diffusion by eddies from a strip. On some of the other gradients, studies were made late enough for secondary spread to have taken place; for these reference should be made to the original paper. One plot which was scored on a series of dates is of interest in showing the progressive damping out of the gradient following local secondary spread of infection (Table 20, Fig. 5 *F*). In several of their tables the authors gave both the percentage of plants attacked and the number of spots on leaves of different ages. Unfortunately, these data cannot be used to test the appropriateness of Thompson's transformation because the two sets of data were not derived from the same groups of plants.

Table 20. *Development of mildew* (Erysiphe graminis) *on field of spring barley at Screvenborn, Kiel, adjoining a field of winter barley* (Pape & Rademacher, 1934)

Distance from source m.	10 May Probable lesion no.	24 May Probable lesion no.	31 May Observed lesion no.
50	102	—	314·4
100	58	187	210·2
150	28	212	167·9
200	45	190	144·1
250	51	255	192·3
300	8	187	135·5
350	8	58	123·1

A gradient from an area source similar to the above is indicated by Gassner (1916), who trapped uredospores of *Puccinia coronata* at various distances in a north-west direction from an oat field in Uruguay. At 5 m. he found an average of 3·4 spores per unit area, at 100 m. 1·8 spores, and at 670 m. 1·9 spores (Fig. 5 *G*).

Gradients from line or strip sources with particles which are outside the scope of the present review, but apparently compatible with the theory, are to be found in data on dispersal of the fruits of *Leontodon* (*Apargia*) *hispidus* (Brownlee, 1911), and the distribution of atmospheric contamination around Norwich (Brunt, 1932).

V. Discussion

In the theory developed here the dilution of the suspended spore cloud by eddies is regarded as the major factor controlling the deposition gradient, and in this respect the theory differs from the usual treatment (e.g. McCubbin, 1944) by relegating the speed of fall of spores and speed of wind to the role of second-order corrections. Horizontal and vertical dispersal are part of the same diffusion process, and the ultimate vertical gradient depends on turbulence and rate of fall. The theory allows for the deposition of large quantities of spores beneath the pileus of an agaric

(Buller, 1909, p. 217) even in exposed situations, and it may be noted here that the deposition of fungus spores in high concentration on a small area is not necessarily wasteful because the phenomenon of hyphal fusions, which permits the vegetative union of mycelia from different spores of the same species, probably leads to the virtual abolition of intraspecific competition among the higher fungi.

It has been shown that the observed gradients of undoubted wind-dispersed spores are compatible with recent theories of eddy diffusion in the atmosphere, and that data on spores dispersed from a point source are in very close agreement with it. It is not possible to predict the absolute number of lesions from a source, as this must depend on how favourable conditions are for infection, but if the intensity of infection is known in any one position it should become possible to predict the numbers of infections at any other distance over a uniform field. The number of lesions at some standard distance might well be taken as a comparative measure of the totality of factors favouring infection. The limits of spore dispersal are theoretically unbounded until the spores are washed out of the air by rain, but the gradients predicted by this theory are nevertheless steeper than those previously deduced by Schmidt, who believed that one-hundredth of the spores of *Bovista* liberated near the ground would travel more than 460,000 km. Interpolation of the table of Q_x (Appendix) indicated that on the present theory only one-hundredth would travel more than 16 km. under normal conditions of turbulence, and with low turbulence the limits of dispersal would be as low as 50 m. In this connexion it is of interest that Hyde and Williams (1944) as a result of daily trapping of pollen for a year near Cardiff concluded that most of the pollen trapped could have originated within 1000 m., and that very little need have come from more than 10 km. As a practical deduction from this, more reliance can be placed on the effect of spatial separation in protecting clean crops from contamination, but more attention should be paid to eliminating sources of infection within the crop itself. In a comparatively little known paper, Fracker (1936) has studied the influence of the distribution of sources of *Cronartium ribicola* within stands of *Pinus Strobus*, and has obtained a measure of the effect of non-uniform distribution of *Ribes* in relation to the distribution of *Pinus*. It was shown that ecological factors causing aggregation of the *Ribes* resulted in fewer infected trees than would be expected from the same amount of *Ribes* uniformly scattered.

The use hitherto of a double logarithmic scale, although convenient in plotting observations over a large range of concentrations and distances, has obscured the essential feature of the curve. This is better shown in Fig. 6, where Bonde and Schultz's (1943) data for potato blight from Table 10, and the theoretical curve for dispersal from a point source ($m = 1{\cdot}75$) are plotted on an ordinary arithmetical scale. This brings out the fact that for a given interval of distance near the source, the deposition changes much more rapidly than over the same interval in a remote position. The gradient is thus a 'hollow curve'. This may help to explain the two apparently distinct forms in which potato blight may appear on a field: spread from a few foci, or evenly spotted over the field, without

invoking splash dispersal for which there is no evidence in *Phytophthora infestans*. In the former case spread is from sources (not necessarily primary) within the crop and the number of lesions decreases steeply a few yards away from the centre. Where the spots are evenly distributed the field probably lacks foci and is located on the flattened tail of the gradients of one or many distant sources of infection.

The study of disease gradients bears on the design of field experiments with epidemic plant diseases. Current designs for replicated and factorial trials have been developed largely for tests with varieties and fertilizers in which it is fair to assume that, provided narrow guard strips between plots are discarded, the treatment given to one plot does not affect its neighbours. With air-borne organisms attacking the shoot systems of plants this assumption is invalid. For instance, a small plot of potatoes sprayed with a copper fungicide for the control of blight might be exposed to many

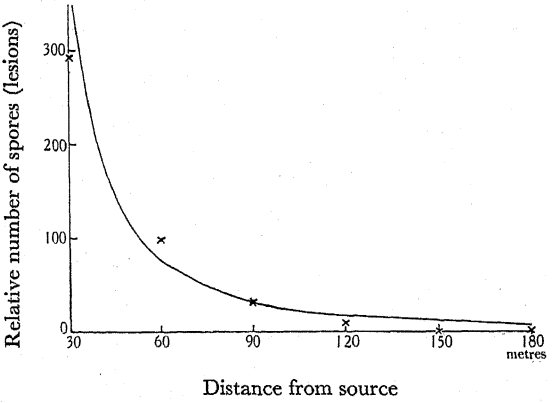

Fig. 6. Expected deposition from a point source (—) plotted on ordinary numerical scale ($m = 1.75$), and observed gradient of potato blight lesions (×) from a point source, Bonde and Schultz's data.

thousands of times the intensity of contamination that it would have experienced if surrounded only by treated plants. The width of guards can be increased only at the cost of increasing error from other causes. One approach to the problem would seem to lie in arranging plots so that the gradient of infection can be taken into the calculation.

The use of gradients as a research tool has already been mentioned in enabling the source of potato-blight outbreaks to be traced by following the gradient to its source. Light may also be thrown on more obscure problems when it is considered that the gradient of passively air-borne spores dealt with in this paper is only one among a number of types of gradient that may be shown by land micro-organisms. The disease gradients due to a fungus extending radially by hyphal growth such as a ringworm lesion on the skin, patches of clover rot (*Sclerotinia trifoliorum*), or tropical species of *Rosellinia* (Nowell, 1923), are exceedingly sharp and characteristic. Slime-spored fungi and most bacterial plant pathogens appear to depend for transport on splashing by raindrops (Faulwetter,

1917; Rolfs, 1942), and this mechanism also plays a part in the dispersal of *Sclerospora* from maize (Weston, 1923). The theory of this dispersal mechanism has not yet been worked out, but from the slight data noted above the gradient appears flatter than that resulting from wind transport alone. This is possibly because, while small water droplets containing spores may themselves be carried like dry spores in the wind, the spore when once deposited on a surface is not permanently out of play but is eligible for a further movement by another rain splash. As Garrett (1943) points out: 'such a mode of dispersal would probably be most effective in increasing the incidence of disease within a crop, but much less effective (than dry air) in transferring it from one crop to another.' The splash mechanism needs special study, both in the open air and under glasshouse conditions where splashing with water may be vigorous.

Still another condition of dispersal obtains when transfer is carried out by insects moving under their own power. The particle dispersed may be a protozoan, pollen grain, fungus spore or virus, but the gradient is likely to be that due to the diffusion of a stationary cloud, not to the wind-blown cloud dealt with in this paper. A study of this mechanism was attempted by Pearson and Blakeman (1906) and by Brownlee (1911), without much progress. Some field data for virus diseases have been given by Frampton and others (1942) and by Bald and Norris (1943). Zentmeyer, Horsfall and Wallace (1943) in their study of Dutch elm disease found that, for the local spread of the slime-spored, insect-borne fungus, *Ceratostomella ulmi*, 'the percentage of trees diseased, plotted as probits, decreased with the logarithm of the distance from a central source of inoculum.' Detailed discussion of these mechanisms must be postponed for the present.

Several kinds of deviation from the theoretical gradients are to be expected. Sampling errors become relatively greater at greater distances, both because of the difficulty of accurately assessing a very low level of infection without taking very large samples, and also because of the comparative rarity of the larger eddies of which a number must have operated before the very distant distribution is smoothed. Given a large number of eddies the dispersion pattern is regular, not in spite of eddies, but because of them. Another source of deviation is the fact that in western Europe and many tropical countries at any rate the uniform topographical field which we have postulated is only realized over very small areas.

Once the general type of gradient has been established for a topographically uniform field it should become possible to study quantitatively the effect of various kinds of natural and artificial barriers, such as screens, intervening crops and topographical features, on the dispersal of spores and pollen (Rempe, 1937; Roemer, 1932; Pope, Simpson & Duncan, 1944).

The dispersal of spores in air is a complex process, and it is not to be expected that the patterns of dispersion would be explained by means of a few aeroplane ascents in North America and Europe, a dozen or so experimental plots in various parts of the world, and two tests on trapping artificially liberated spores in the open air at Leningrad. The formulae provisionally suggested need not therefore be taken too seriously, but the

facts reviewed here indicate that viewed on a logarithmic scale there is an orderliness about the process which was perhaps unexpected, that the range of density of deposition varies with distance from the source within fairly narrow limits, and that out of the multitude of factors affecting the deposition at any distance the strength and the dimensions of the source and the turbulence of the atmosphere are predominant.

This interpretation of observed gradients is suggested tentatively as a first approximation in the hope of evoking more experiments and better field records in this aspect of aerobiology.

Summary

The deposition of air plankton, such as passively air-borne pollen grains or fungus spores, decreases with increasing distance from a source. The factors controlling the scattering of air plankton are reviewed, and observed gradients of deposition are discussed with special reference to fungi causing diseases of plants.

The terminal velocities of spores are shown to depend on their dimensions and to be roughly of the order expected for smooth, spherical particles from Stokes's law, but fairly wide deviations between observed and expected values are believed due to surface roughness and asymmetry. Fungus spores have been observed to fall with velocities between 0·04 and 2·5 cm.sec., and pollen grains between 1·5 and 40 cm.sec. The mean wind velocity at 10 m. is near 300 cm.sec. Attempts to calculate the dispersal of spores as the resultant of vertical fall under gravity and horizontal wind movement are shown to apply only to non-turbulent air movement, and therefore to be inapplicable at heights more than a few millimetres above the earth's surface. Differences in rate of spore fall are probably not major factors in dispersal. It is more appropriate to consider a spore cloud in suspension in the air in process of being diluted by eddies in the course of its transport by the wind.

In support of the concept of the spore cloud as a suspension it is shown that at heights in the atmosphere above the surface layers, the concentrations of pollen and spores decreases exponentially with increasing height in the manner that would be expected if particles falling under gravity were balanced by other particles diffused upwards by eddies. The values for eddy diffusivity, K, varying from $1{\cdot}5 \times 10^4$ to $3{\cdot}3 \times 10^5$, deduced for spores and pollen, are of the order usually found in meteorological work.

While terminal velocity may play only a small part in spore dispersal, it may be more important in causing deposition of spores brought down by eddies to the boundary layer of relatively still air a few centimetres thick at the earth's surface.

The earlier theories of eddy diffusion, including that of Schmidt, are compared with recent work by Sutton who considered that the size of eddies effective in diluting a cloud, instead of remaining constant, increases with the distance travelled by the cloud. Experiments by Stepanov, in which spores were liberated from a point in the open air and trapped at various distances and in different directions, are shown to be in excellent

agreement with Sutton's theory, and to lead to almost identical values for the parameters for diffusion and turbulence with those found by Sutton.

Based on Sutton's theory, equations are given for the deposition of spores at various distances from a point source, and from Stepanov's data it is concluded that in travelling across 1 sq.cm. of surface there were deposited the equivalent of the number of spores present in a layer on the axis of the cloud about half a millimetre thick. This value is expressed as a coefficient of deposition, p, and it is regarded as a parameter of considerable biological significance.

The relevance of the deposition formula is discussed. Observed gradients of air-borne plant infections originating from a point source are shown to be closely predicted by this theory. Fungi known or suspected of being splash-dispersed on the contrary show gradients incompatible with the theory. Gradients from strip sources cannot be dealt with satisfactorily at present, but an approximate formula is given, and observed gradients from strip sources are found to show reasonable agreement.

The significance for plant hygiene of this interpretation of fungus spore dispersal is that, while attention should be paid to isolation, most emphasis should be placed on eliminating foci of disease within a crop.

This work suggested itself during the course of field studies on potato-virus diseases carried out under the auspices of the Agricultural Research Council. Thanks are offered to my colleagues and correspondents who have been generous with advice and suggestions during the course of the study, and especially to my wife, Margaret F. Gregory, who contributed the Appendix and whose mathematical help has been invaluable. None of these, however, is responsible for the interpretation of the data suggested here.

APPENDIX

(1) *The number (N) of spores in a slice of cloud*

The number of spores in a slice through the spore cloud in the xOy plane 1 cm. thick is found by integrating the number of spores in an annular element of this slice between radii r and $(r+\delta r)$. The limits are from 0 to ∞ as the cloud has no finite boundary:

$$\begin{aligned}
N &= \int_0^\infty \chi 2\pi r\,dr \\
&= \int_0^\infty \frac{2Qe^{-(r^2/C^2x^m)}}{\pi^{\frac{1}{2}}C^3x^{\frac{3}{2}m}}\,2\pi r\,dr \\
&= \frac{2Q}{\pi^{\frac{1}{2}}C^3x^{\frac{3}{2}m}}\int_0^\infty e^{-(r^2/C^2x^m)}\,2r\,dr \\
&= \frac{2Q}{\pi^{\frac{1}{2}}C^3x^{\frac{3}{2}m}}\left[-C^2x^m e^{-(r^2/C^2x^m)}\right]_0^\infty \\
&= \frac{2Q}{\pi^{\frac{1}{2}}Cx^{\frac{1}{2}m}}.
\end{aligned}$$

(2) Q_x, *the quantity of spores in the cloud when its centre has moved a distance* x

Deposition, $$D = pN = \frac{p2Q}{\sqrt{(\pi)}\, Cx^{\frac{1}{2}m}}.$$

Assume the rate of deposit uniform while the cloud moves a distance δx. Then the deposit during this move

$$(-\delta Q) = \frac{p2Q}{\sqrt{(\pi)}\, Cx^{\frac{1}{2}m}}\, dx,$$

$$\frac{dQ}{Q} = -\frac{2p}{\sqrt{(\pi)}\, C}\frac{dx}{x^{\frac{1}{2}m}}.$$

The solution of this equation is

$$Q_x = Q_0 \exp\left[-\frac{2px^{(1-\frac{1}{2}m)}}{\sqrt{(\pi)}\, C(1-\frac{1}{2}m)}\right].$$

(3) *Deposition down wind* (d_w) *from a point source at the origin*

Take the x-axis in the direction of the wind and consider a cylinder of unit cross-section around this axis. The number of spores (n_w) passing over the point $(x, 0, 0)$ is the total number in this cylinder. The number of spores in an element of the cylinder length δr is $1\delta r\chi$. Therefore

$$N = \int_{-\infty}^{\infty} 1\, dr\chi$$

$$= \int_{-\infty}^{\infty} \frac{2Qe^{-(r^2/C^2x^m)}\, dr}{\pi^{\frac{1}{2}}C^3x^{\frac{1}{2}m}}$$

$$= \frac{2Q}{\pi^{\frac{1}{2}}C^3x^{\frac{1}{2}m}} \int_{-\infty}^{\infty} e^{-(r^2/C^2x^m)}\, dr$$

$$= \frac{2Q}{\pi^{\frac{1}{2}}C^2x^m}\sqrt{\pi}$$

$$= \frac{2Q}{\pi C^2x^m},$$

$$d_w = \frac{p2Q}{\pi C^2x^m}.$$

(4) *Number* (d_{lw}) *of spores from a line source deposited down wind at right angles to the line only*

The spores are emitted from the y-axis. The number of spores (Q_0) emitted at the point $(0, y, 0)$ passing through an element of volume at a point $P(x, 0, 0)$ is the same as the number of spores from a puff Q emitted at the origin passing through an element of volume at $(x, y, 0)$. Thus we

can consider each puff as coming from the origin and passing over elements of the strip parallel to Oy through P. The number of spores passing over this strip is that in a slice of cloud Q emitted from the origin and this has already been shown to be

$$\frac{2Q}{\sqrt{(\pi)Cx^{\frac{1}{2}m}}},$$

therefore

$$d_{lw} = \frac{p2Q}{\sqrt{(\pi)Cx^{\frac{1}{2}m}}}.$$

Table 21. *Quantity of spores in the cloud and deposition per unit area*

Calculated for: $Q_0 = 10^{10}$; $C = 0{\cdot}6$; $2p = 0{\cdot}1$

	Distance in cm.									
	10	100	1000	2000	5000	10,000	20,000	50,000	100,000	1,000,000
$\log_{10} x$	1·0000	2·0000	3·0000	3·3010	3·6990	4·0000	4·3010	4·6990	5·0000	6·0000
$\log_{10} Q_x$										
$m = 1{\cdot}75$	9·5761	9·4282	9·2289	—	—	8·9600	—	—	8·5960	8·1070
$m = 1{\cdot}24$	9·7422	9·3816	8·5170	8·1980	7·2660	6·4410	5·3690	3·4400	—	—
$\log_{10} D$										
$m = 1{\cdot}75$	7·6794	6·6615	5·5922	—	—	4·4533	—	—	3·2193	1·8603
$m = 1{\cdot}24$	8·0955	7·1149	5·6303	5·1247	3·9460	2·9343	1·6757	$\bar{1}{\cdot}5000$	—	—
$\log_{10} d$										
$m = 1{\cdot}75$	5·8813	3·8634	1·7941	—	—	$\bar{1}{\cdot}6552$	—	—	$\bar{3}{\cdot}4212$	$\bar{5}{\cdot}0622$
$m = 1{\cdot}24$	6·2974	4·3168	1·8322	1·0256	$\bar{1}{\cdot}4489$	$\bar{2}{\cdot}1362$	$\bar{4}{\cdot}5766$	$\bar{6}{\cdot}0029$	—	—
$\log_{10} d_w$										
$m = 1{\cdot}75$	7·0736	5·1757	3·2264	—	—	1·2075	—	—	$\bar{1}{\cdot}0935$	$\bar{4}{\cdot}8545$
$m = 1{\cdot}24$	7·7497	6·1491	4·0445	3·3523	1·9267	0·7285	$\bar{1}{\cdot}2833$	$\bar{4}{\cdot}8607$	—	—

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ANNUAL GENERAL MEETING

9 December 1944

The Annual General Meeting for 1944 was held in the Department of Biology, Chelsea Polytechnic, London, on Saturday, 9 December, with the President, Mr R. W. Marsh, in the Chair.

After the Minutes of the last Annual Meeting had been read and signed, the President announced with deep regret the deaths of six members: Miss D. Ashworth, Prof. A. H. R. Buller, Miss C. A. Cooper, Mr R. A. Findlayson (who died in 1943), Mr A. W. Oke, and Mr A. Wallis.

Reviewing the past year the President recalled that the East Malling meeting had to be cancelled, but that the other meetings and a successful series of day forays had been held as arranged. He announced that the Council had, that morning, established a permanent Foray Committee of four members (one to retire each year) together with the Secretary *ex officio*, to organize the Society's field activities. He drew attention to the publication of the third edition of the *List of Common Names of British Plant Diseases* under the new title of *List of Common British Plant Diseases* (a copy of which had been sent to every member), expressing the Society's thanks to the Agricultural Research Council for a grant of £100 towards the cost of publication and to Mr W. C. Moore and Dr G. R. Bisby for editing this co-operative work and seeing it through the press. He also reported that in June a Sub-committee consisting of K. St G. Cartwright, R. V. Harris, W. C. Moore (*Chairman*), G. Smith, Miss E. M. Wakefield (*Secretary*), and the President and Secretaries *ex officio*, which had been appointed by the Council in October 1942 to investigate the present state of systematic mycology in this country and the part the Society might play in its development, presented a comprehensive report to the Council. The Council decided that the report should not be published at present, but it has been printed and copies have been sent to all Universities, Government Departments, Institutes, and Associations concerned with the activities of fungi. Attention was also drawn to the collection of surplus reprints on mycology and plant pathology for war-damaged libraries which the Society was organizing.

After the year's Accounts had been submitted by the Treasurer, Mr A. A. Pearson, and adopted, the following Officers and Councillors for 1945 were elected: *President*, G. Smith, M.Sc.; *Vice-Presidents*, Mrs E. W. Mason and the two past-presidents, R. W. Marsh and S. P. Wiltshire; *General Secretary*, J. Ramsbottom; *Secretary*, G. C. Ainsworth; *Treasurer*, A. A. Pearson; *Editors*, B. Barnes and H. Wormald; *Councillors*, R. W. G. Dennis, C. G. Dobbs, and G. Samuel (to replace S. D. Garrett, Mrs E. W. Mason and A. Smith). The Plant Pathology Committee's nominations of H. E. Croxall, W. C. Moore and E. R. Wallace (to replace W. Buddin, W. R. Day and A. Smith) were agreed to. Six new members were elected, making twenty-two for the year.

The changes in the Rules which had been proposed by the Council so that the Society could accept Associates were then discussed and it was unanimously agreed that:

Rule 4 shall be deleted and that Rules 2 and 11 shall read:

2. The Society shall consist of Honorary Members, Members (including Foundation Members*), and Associates; the number of Honorary Members shall be limited to 20 at any one time but the numbers of Members and Associates shall be unlimited.

*Foundation Members are those Members or Societies who joined the Society previous to the limit of 100 Members having been attained. This was reached 22 October 1903.

11. The Society shall hold one or more meetings annually at a place and time determined by the Members at the preceding Annual General Meeting, or by the Council.

and that this new Rule be inserted after Rule 10:

All Associates shall be proposed by two Members. They shall be elected by a majority of the Members present at a meeting of the Society and shall pay an annual subscription of five shillings. They shall be eligible to attend the Society's meetings and forays but not to vote at any meetings or to receive the *Transactions*.

After discussing the programme for 1945 the meeting adjourned until 2 o'clock when the President delivered his address entitled 'Mycological Contacts'. A vote of thanks to Mr Marsh concluded the meeting.

G. C. AINSWORTH
Secretary

RECEIPTS AND PAYMENTS

for the year ending 30 June 1944

1943		£	s.	d.
1 July	Balance brought forward ...	240	8	11
4 Aug.	Grant from Royal Society ...	100	0	0
1944				
22 March	Grant from Agric. Research Council	100	0	0
3 May	Sale of *Transactions*:			
	Current Volume	104	14	0
	Back Volumes	71	5	8
	Sale of Reprints	5	7	10
	Sale of			
	British Basidiomycetae ...	4	8	7
	Diseases of British Grasses ...	2	13	3
30 June	Subscriptions to date ...	335	2	4
	Gifts to Printing Fund ...	4	0	0
	Deposit Interest	1	0	0
	War Loans Interest	25	14	4
		£994	14	11

1944		£	s.	d.
3 May	Cambridge University Press: Cost of Vol. XXVI *Transactions* and Reprints ...	421	0	6
30 June	Postages, etc.			
	Treasurer	3	0	0
	Secretary	11	11	5
	Sec. Path. Committee ...	1	9	0
	Editor	1	17	7
	Sundry Printing	10	13	6
	Fees of Meetings	3	19	6
	Purchase of *Transactions* ...	8	2	6
	Honorarium to Miss Kierans	4	4	0
	Photograph of Berkeley Portrait	2	13	0
	Balance in bank	526	3	11
		£994	14	11

CAPITAL ACCOUNT—*Robinson Bequest* £50, *Cheesman Fund* £150, *Printing Fund* £500 } *invested in* £255 3½ % *War Stock.* £245 3½ % *War Loan.* £200 3 % *Defence Bonds.*

A. A. PEARSON, Hon. Treasurer

Examined and found correct, E. W. SWANTON

FORAYS, 1944

A spring foray was held at Ruislip woods on 20 May, and a series of day forays was arranged for the autumn on the same lines as in 1943. The Glasgow and Andersonian Natural History and Microscopical Society (9, 16, 30 Sept., 7 Oct.), the Haslemere Natural History Society (16 Sept.), the Yorkshire Naturalists' Union (22–26 Sept.), the Norfolk and Norwich Naturalists' Society (28 Sept., 8, 21–22 Oct.), the Bournemouth Natural Science Society (5, 26 Oct.), the Leicester Literary and Philosophical Society (Botany and Biology Section) (7 Oct.), and the Lincoln Naturalists' Union kindly invited members to attend their forays. Joint forays were held with the Hertfordshire Natural History Society (16 Sept.), the Chingford Branch (23 Sept.) and the Ecological Section (29 Oct.) of the London Natural History Society, the South London Botanical Institute (Oxshott, 7 Oct.), and the Maidstone Scientific and Antiquarian Society (East Malling, 21 Oct.), while Ruislip woods (1 Oct.) and Englefield Green (14 Oct.) were again visited by the Society.

At the first of the joint forays, a large party visited Whippendell woods near Watford where the commoner species were found to be in good variety. In Epping Forest on 23 Sept. the Society began a census of the fungi in the Cuckoo Pits area where the Chingford Branch of the London Natural History Society are making a detailed ecological survey and later in the season assistance was also given to the Ecological Section of the same Society with its Bookham Common survey. Ruislip woods again provided a rich harvest when Mad Bess wood was worked this year for the first time. Among the finds was *Boletus cramesinus* Secr. (=*B. sanguineus* Wither. ex Fr. var. *gentilis* Quél.) (specimens of which have been deposited in the Kew Herbarium), for which there appears to be no published British record, although it was found by E. W. Swanton and A. A. Pearson at Shillinglee Park, Sussex, in 1943; and two very large sporophores of *Psalliota augusta* from a nearby allotment attracted attention. The Englefield Green foray added further records to those of previous years for the Clockcase plantation, and at East Malling specimens of over 100 species were laid out in the laboratories of the Research Station to which the Society was indebted for hospitality. A feature of the latter foray was an interesting collection of species of *Clavaria* comprising *C. cinerea, C. corniculata, C. cristata, C. inaequalis, C. Kunzei, C. rugosa,* and *C. stricta.*

The Forge Valley area covered by the Yorkshire Naturalists was good fungus country and nearly 250 species were recorded. Yelmandale and Bedale woods and the Raincliffe woods were visited and some interesting associations were noted by Miss Grainger. *Hygrophorus eburneus,* dominant on the dry limestone slopes under beech, was associated with several species of *Cortinarius,* e.g. *C. purpurascens* and *C. testaceus. Psalliota xanthoderma* var. *obscurata* Maire was found in pastures near Ayton. (There is no published record of this fungus in Britain although it has been gathered in the southern counties.) *Lactarius deliciosus* and *Lycoperdon depressum* were abundant in the spruce plantings in Raincliffe wood. On the Saturday, Mr A. A. Pearson, Chairman of the Mycological Committee of the Y.N.U., gave an address on 'The study of agarics'.

The last foray of the season was the joint one with the London Natural History Society to Bookham Common where a survey of the flora and fauna was begun in 1941. As a result of this year's visit the list of the fungi of this area was increased to 180 species. The woods are mainly oak and the only specimen of *Amanita muscaria* found was under a solitary birch.

UNA C. MASON
Secretary of the Foray Committee

PROCEEDINGS

Meeting held in the English Lecture Theatre, the University, Edmund Street, Birmingham, 28 October 1944. The President, R. W. Marsh, M.A., in the Chair.

Seed-borne fungi

A. SMITH. Seed examination at the Plant Pathology Laboratory, Harpenden.
Miss MARY NOBLE. *Phialea temulenta*—the Blind Seed fungus.
Miss K. SAMPSON. Some endophytic fungi of grasses.
W. A. MILLARD. Broccoli Canker.
L. G. G. WARNE. A seed-borne outbreak of Club Root.
G. C. AINSWORTH. The geographical distribution of some seed-borne fungi.

General Discussion. [For a summary of this discussion, see *Nature, Lond.*, CLV (3924), 36–7, 1945.]

Vol. XXVIII, Parts III & IV November 1945

OBSERVATIONS ON THE PERENNIAL CANKER FUNGUS

GLOEOSPORIUM PERENNANS ZELLER & CHILDS

By E. H. WILKINSON, *Research Station, Long Ashton, Bristol*

(With Plate II and 3 Text-figures)

1. Introductory

A preliminary note (Wilkinson, 1943) recorded that a fungus frequently isolated from dieback and canker lesions of apple shoots and from storage rots of apple fruits in England was identical with *Gloeosporium perennans* Zeller & Childs, described in the U.S.A. as the cause of the apple disease known as Perennial Canker. That identification is confirmed here and further details are given of the isolation, cultural characteristics and pathogenicity of the fungus, in comparison with the American findings.

The disease caused by *G. perennans* in the U.S.A. was termed Perennial Canker because the lesions produced could increase in size for a period of six or seven years with the production of successive concentric rings of callus. Subsequent work (Childs, 1929; Cooley & Shear, 1931; Brown, 1932; McLarty, 1933) has shown that annual extensions of the canker do not occur in the U.S.A. unless the limiting callus layer is injured by low temperatures or Woolly Aphis attack. In England it has been shown (Wilkinson, 1944) that the most serious phase of the attack by *G. perennans* is the dieback of summer-pruned trees, and that the cankers formed are normally of the restricted type with a single callus layer. Exceptionally, two rings of callus are formed, following Woolly Aphis injury. Further work on the conditions determining extension of the cankers is described here.

Cross-inoculations show that *G. perennans* isolated from shoots causes a rot of apple fruits in this country. This apple rot is not distinguishable by superficial examination from that caused by *G. album* and it was suggested (Wilkinson, 1944) that the disease in the field should be included under the term Bitter Rot. Comparisons are made below of the lesions caused on apples by *G. album*, *G. fructigenum* and *G. perennans*.

2. Identity and cultural characteristics of the pathogen

Zeller and Childs named the fungus causing Perennial Canker *Gloeosporium perennans*, having 'conidia hyaline, irregular in shape and size, mostly ellipsoidal, usually larger at one end, seldom slightly curved, 12–20 × 4–6 μ; producing secondary conidia in culture; secondary conidia hyaline, variable in size, 3–10 × 1–2 μ, ovoid to ellipsoid, sometimes curved'.

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A description of Perennial Canker as it occurs in England and the pomological aspect of the attack have been given in a previous publication (Wilkinson, 1944).

Sporing material of the fungus, isolated by the author from infected branches and fruits in this country, was obtained by incubating infected branches and apples in moist chambers at 21° C. for three days. Spore suspensions from the two sources were made and plated out on 2 % malt-extract agar from which monoconidial isolations were obtained. The isolations, whether from cankers or apple rots, collected from various parts of the country, were similar. Initially, a small tuft of white mycelium is formed which grows slowly, the thallus being little more than 1 in. in diameter after twenty-three days. At first the mycelium turns grey, then a delicate pink, but eventually becomes dirty white and assumes a ragged woolly appearance. In plate cultures, the marginal growth is submerged; no cracking of the agar occurs, but a brown discoloration is evident when the cultures are a few days old. In tube cultures the fungus produces black apothecium-like bodies on the underside of the agar slope; they are compressed against the glass wall of the culture tube and cause the lifting of the agar cylinder. No unmistakable asci or ascospores have been observed. Similar incipient apothecia were described by Kienholz (1939) who also reported the discovery of the perfect, apothecial, stage of *G. perennans* occurring naturally on apple tree cankers; this he named *Neofabraea perennans*.

Acervuli appear slowly when a single isolation is grown on plate cultures, but their formation is appreciably accelerated when eight to ten colonies are grown on the same plate. They are formed irregularly on the agar surface, arising mainly from the submerged marginal mycelium or over the surface of the aerial mycelium. The former arise as small masses of compact, dark, undifferentiated hyphae, the sporing layer being distinctly concave, whereas those formed on the surface of the thallus are frequently found at the distal end of comparatively long necks of sterile, hyaline hyphae.

The conidiophores are erect, branched, hyaline, septate, approximately three to four times as long as the spores, the latter being budded off terminally and successively, and accumulating as small white masses when young, but tending to become pale yellow with age. The conidia are non-septate, hyaline, irregular in size, straight, usually larger at one end and mainly ellipsoidal measuring 10·5–18·7 × 3–6 μ (Text-fig. 1 *b*).

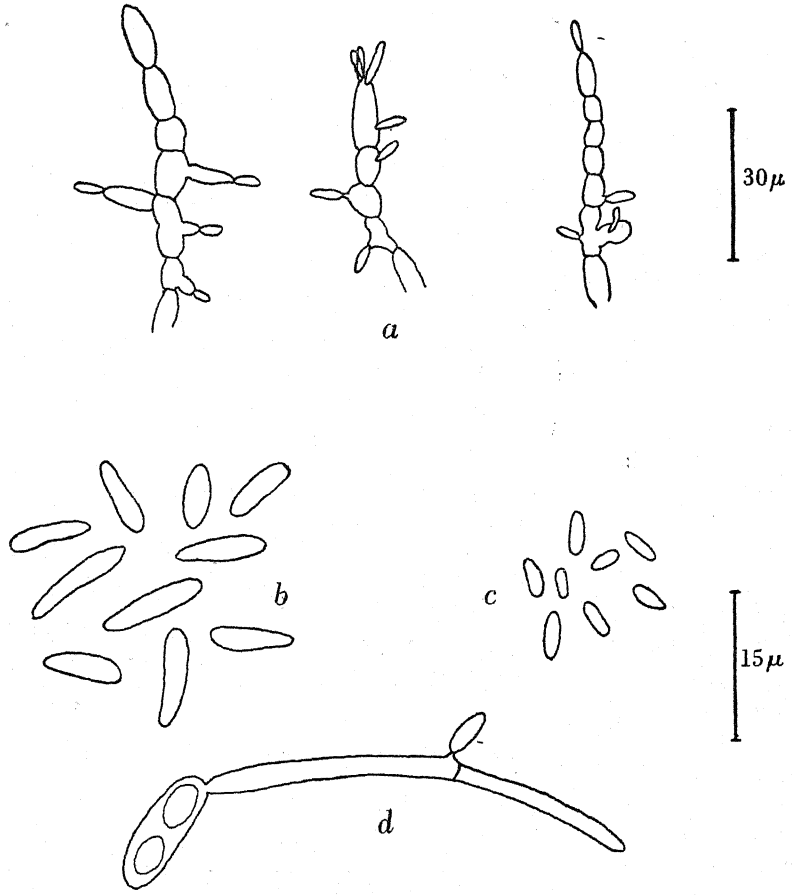
Conidia produced on 2 % malt-extract agar by isolations of the fungus from apple fruits and branch infections measured 4·5–21 × 1·5–6 μ. This range is greater than that given by Zeller and Childs as secondary conidia, which were readily produced in culture, are included.

Formation of secondary conidia

G. perennans forms secondary conidia in culture and aqueous nutrient solutions, a fact reported by Zeller and Childs (1925), but in addition I have observed them on natural lenticel rots and branch infections. Using spores taken from a culture of the fungus isolated from a lenticel

rot of Laxton's Superb, their formation at laboratory temperatures was followed in hanging drops of sterile distilled water and 0·1 % sucrose solution and also on 2 % malt-extract agar.

In the hanging drops, germination occurred readily after five hours, the germ-tubes originating at the ends of the spores, a central septum being formed when two developed from the same spore. Very occasionally a



Text-fig. 1. Formation of secondary conidia in *G. perennans*. (*a*) On 2 % malt-extract agar. (*b*) Normal conidia from Bitter Rot lesion. (*c*) Secondary conidia formed in 0.1 % sucrose solution. (*d*) Budding-off of secondary conidium in 0.1 % sucrose solution.

germ-tube is produced on the side close to the septum. In sterile distilled water secondary conidia were formed after seventy hours, and in 0·1 % sucrose solution after forty-seven hours. Each secondary conidium is produced independently, but successively, from a point immediately below each septum of the germ-tubes and when liberated floats away, when the formation of another begins (Text-fig. 1*d*). In both solutions they are small, hyaline, non-septate, ellipsoidal (Text-fig. 1*c*) measuring 3·0–6·0

× 1·5 μ (mean 5·0 × 1·5 μ) in sterile distilled water, and 3·0–9·0 × 1·5–3·0 μ (mean 5·9 × 1·87 μ) in 0·1 % sucrose solution. The secondary conidia failed to germinate in either solution over a period of five days. Zeller and Childs (1925) give measurements of 3–6 × 1–1·5 μ.

On 2 % malt-extract agar the spores germinated readily in a manner similar to that observed in aqueous solutions. At an early stage the germ-tubes became closely septate and formed numerous branches. After forty-seven hours secondary conidia were produced in large numbers at the apex, and from points immediately below the septa, of the germ-tubes, later becoming detached and adhering in masses around the hyphal growth (Text-fig. 1 *a*). They measured 6·0–9·0 × 1·5–2·25 μ (mean 7·6 × 2·0 μ). As the colony increased in size so also did the size of the conidia liberated; in fact after seventy hours normal conidia were being budded off which in their turn germinated and had produced a further crop of secondary spores after 120 hours. Secondary spores were never seen to germinate. Some spores having one or two septa were also observed which may have originated from fragmentation of the mycelial growth. Hence when a spore of *G. perennans* germinates on malt-extract agar a small amount of hyphal growth becomes surrounded by large numbers of secondary and normal conidia after five days. Eventually the hyphae grow out radially and no further spore formation on these extensions is observed after seven days.

The fungus isolated by me thus agrees closely with the original description of Zeller and Childs. The range in size of the normal spores is about the same as that given by them, while the formation of secondary conidia in nutrient solutions is identical with that described by Zeller and Childs and the size range corresponds very closely. Black apothecial-like bodies in culture described by Kienholz (1939) were also observed in my isolations.

My isolation matches none of the British species of *Gloeosporium* listed (Grove, 1937), but it agrees with Zeller and Child's description of *G. perennans*.

3. Pathogenicity

Cross inoculations

(*a*) *Bitter Rot fungus into branches.* A fourteen-day-old isolation of *G. perennans* from a lenticel rot of Allington Pippin and trees of the varieties Bramley's Seedling, Cox's Orange Pippin, Laxton's Superb and Worcester Pearmain were used for these experiments. On 9 May 1941, four inoculations were made on each variety by cutting a side branch flush with its stouter member and placing a square of inoculum with the mycelium in contact with the cut surface, the operation being completed by binding the wounds with a crêpe-rubber bandage. These were removed one month later when eleven out of sixteen inoculations were showing a marked swelling, accompanied by the splitting and peeling of the bark, 1 in. above and below the point of inoculation. These symptoms became more pronounced, and after ten weeks, distinct cankers had been formed from all sixteen inoculations. They varied from small, black, flattened lesions

on Bramley's Seedling (Pl. II, fig. 1) to those on Cox's Orange Pippin which were 3–4 in. long, showing extensive peeling of the bark, and completely girdling the branches.

In July the bark over each canker became raised at many points producing a blistered appearance. Each swelling finally burst to expose numerous, grey coloured acervuli which had originated sub-epidermally on the cortical tissues. Under humid conditions they liberated small white masses of spores, and re-isolation from these gave pure cultures of *G. perennans*. During the autumn of the same year canker development was arrested by the formation of a callus barrier which isolated completely the diseased from the healthy tissues. Examination in the autumns of 1942 and 1943 showed no further extension beyond this barrier.

A similar series of inoculations was made on 11 November 1941. Cankers 2–3 in. in length were formed by the end of the year when development slowed down presumably as the result of lower temperatures, no callus barrier being formed. The cankers developed slightly toward the end of March 1942, but by April the twelve cankers on three apple varieties had become completely callused off. The lesions on Laxton's Superb continued to expand until midsummer when they, in their turn, were isolated from healthy tissues by callus barriers. Exposed acervuli, liberating small white masses of spores were observed on most of the cankers in May 1942.

Inoculations on uninjured bark of three-year-old branches of the above four apple varieties were made on 9 May and 11 November, but no cankers were formed.

The cankers which developed as the result of inoculations carried out on 11 November 1941, were used in the following experiment in September 1942: Two of the four cankers on each variety were slightly injured by scraping the callus layer with a sterile needle, the remaining two being left uninjured. All cankers were bound with crêpe-rubber bandages which were removed one month later. Extensions of the original lesions developed on six out of the eight wounded cankers—those on Bramley's Seedling failing to show any additional increase—to the extent of 1 in. but these in their turn were callused off by April 1943. Those cankers left unwounded failed to show any further enlargement.

(*b*) *Perennial Canker fungus into apples.* Apples of the variety Allington Pippin were surface-sterilized and inoculated with mycelium from a ten-day-old culture of *G. perennans* isolated from a canker of Early Victoria and retained in moist chambers at laboratory temperatures. Rotting proceeded in a radial manner, forming circular sunken lesions mostly having a dark brown marginal zone surrounding a central pale brown area. The rate of spread was slow, one rot being 1½ in. in diameter after thirty-seven days. Sporing bodies, originating subepidermally, appeared after twenty-five days as small blisters on the surface of the rots, finally rupturing the skin to become exposed as grey acervuli liberating small white masses of spores. Re-isolation from both spores and rotted tissues yielded pure cultures of *G. perennans*.

Similar rots were produced when apples were inoculated with *G. perennans*

isolated from dieback lesions of Cox's Orange Pippin and Laxton's Superb.

To investigate whether *G. perennans* from branch infections would infect apples through lenticels or uninjured skin the following experiment was performed. A Bramley's Seedling fruit was surface sterilized and ten small glass tubes were attached perpendicularly to its surface with medicinal vaseline so that a single lenticel was enclosed by the attached end of each tube. A second fruit was treated similarly, but the tubes were placed over unbroken skin with no lenticels present. A small volume of a conidial suspension from a culture of *G. perennans*, isolated from a canker of Early Victoria, was pipetted into each tube so that no air lock was formed between the suspension and the apple skin. The tubes were then sealed with cotton wool plugs. After five days the tubes were removed and the apples kept under observation. The fruit with the inoculated lenticels developed ten small circular lesions each producing numbers of sporing bodies from which the fungus was re-isolated in a pure state (Pl. II, fig. 2). The fruit inoculated on the unbroken skin failed to produce any rots.

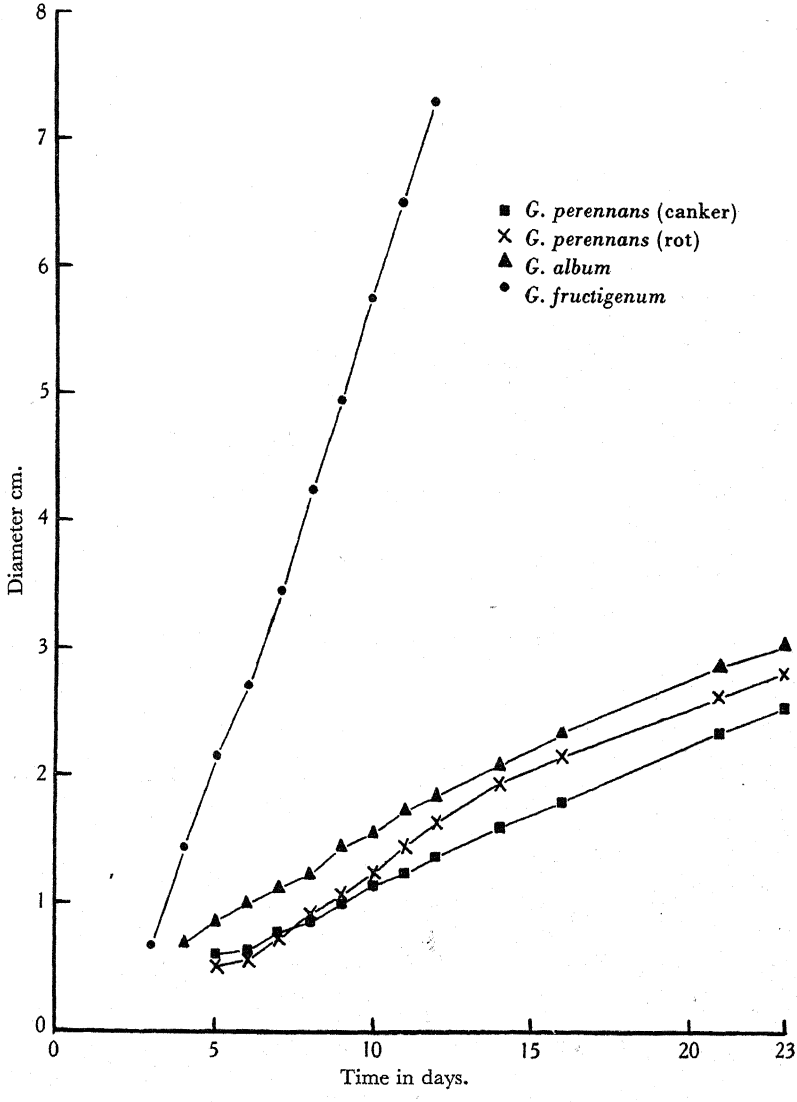
4. Comparison of fungi causing Bitter Rot of apples

Bull's Eye Rot (Fisher, 1925) and Delicious Spots (Brien, 1932) are names given to the fruit rot caused by *G. perennans* in the U.S.A. and New Zealand respectively. In England, however, this apple rot is not distinguishable by superficial examination from Bitter Rot caused by *G. album* Osterw. It was suggested (Wilkinson, 1944) therefore that it should also be included under the name Bitter Rot, thus adding a third species of *Gloeosporium* to the two already known to cause this disease.

Apples inoculated with *G. album* and *G. perennans* produce rots typically having a pale brown or yellow centre surrounded by a darker brown marginal zone with a lenticel usually situated in the middle of each rot. The rate of development of the lesions is nearly identical and sporing bodies appear after twenty to twenty-five days. The acervuli of both fungi burst through the skin to become exposed as small grey bodies which, under moist conditions, liberate white masses of spores embedded in mucilage. In both types of rots, spores have been observed issuing from the acervuli as thin white tendrils. The acervuli are more frequently scattered irregularly over the surface of the rots but are occasionally arranged concentrically especially on those caused by *G. album*.

Separation of the two pathogens by these visual symptoms is not possible and can only be made by examination of the spores which are quite distinct. Those of *G. album* are hyaline, non-septate but distinctly curved, measuring $12–27 \times 4–5\,\mu$, whereas those of *G. perennans* are hyaline, non-septate and mainly ellipsoidal, measuring $4{\cdot}5–18 \times 2{\cdot}2–6\,\mu$. In contrast, the rot caused by *G. fructigenum* is readily distinguished by the more rapid rotting, the lesions being darker and characterized by the production of greyish black surface mycelium and glutinous masses of spores which are ochraceous-buff to *o*-orange (Ridgway, 1912). In addition, inoculations

made by me indicate that *G. album* and *G. fructigenum* are both non-parasitic, whereas *G. perennans* is an active wound parasite, on apple branches.

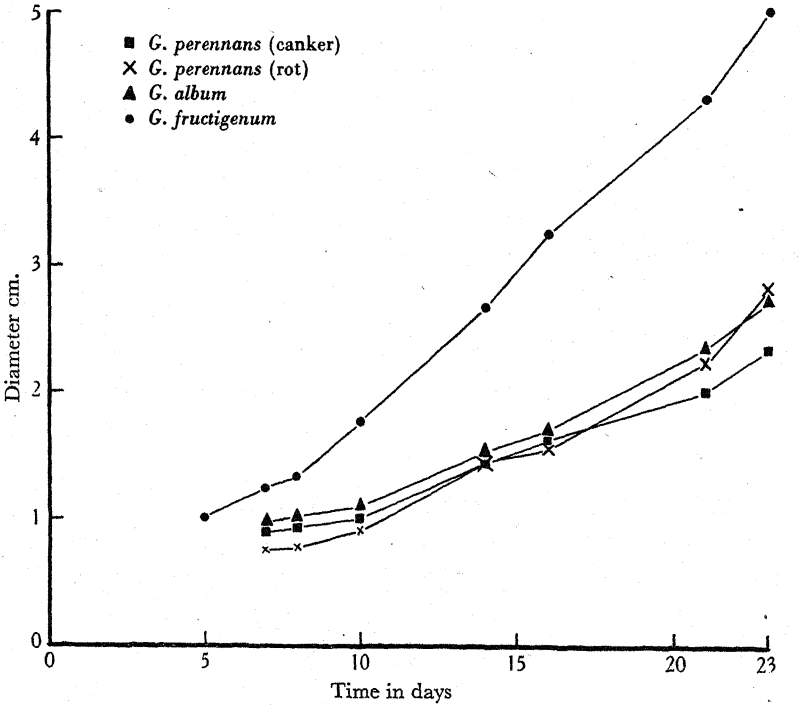


Text-fig. 2. Growth rate of *G. perennans*, *G. fructigenum* and *G. album* on 2% malt-extract agar at 22° C.

The rate of growth in culture of *G. perennans* from branch and fruit lesions and the rate of decay it causes in the fruit were compared with those of *G. album* and *G. fructigenum* under identical conditions using monoconidial isolations.

(a) In culture

Pieces of inoculum 0·2 cm.sq. were cut from the margins of fourteen-day-old cultures of the four isolations and placed in the centre of 2 % malt-extract agar plates. Eight plates were inoculated with each fungus and kept at 22° C. The diameter of the mycelial growth was measured daily over a period of twenty-three days. The results are given in Text-fig. 2 each point representing the mean of eight diameters.



Text-fig. 3. Rate of rotting of *G. perennans*, *G. fructigenum* and *G. album* in Bramley's Seedling at 22° C.

(b) In apples

Sixteen fruits of the variety Bramley's Seedling were immersed in 1/1000 $HgCl_2$ solution for five minutes, washed in running water for ten minutes, dipped in absolute alcohol, and flamed. Each fungus was inoculated into four apples in two places by bringing back a flap of skin and inserting a piece of inoculum 0·2 cm.sq. taken from fourteen-day-old cultures. The skin flaps were replaced and sealed in position by a thin layer of medicinal vaseline, the apples being placed in moist chambers at 22° C. and daily measurements of the diameters of the rots taken over a period of twenty-three days (see Text-fig. 3), each point again representing the mean of eight diameters.

Fig. 1

Fig. 2

5. Summary

1. A fungus isolated from canker and dieback of branches and apple rots in this country is shown to be *Gloeosporium perennans* Zeller & Childs.

2. Cross inoculations demonstrated the identity of the fungus causing branch lesions with that infecting fruits.

3. Some morphological characteristics of *G. perennans* are described.

4. Controlled infection experiments, show that *G. perennans* is an active wound parasite of apple branches, but is unable to penetrate uninjured bark; it cannot penetrate the unbroken skin of the fruit but can traverse the skin through a lenticel.

5. The rate of growth of the fungus and the rate of decay it causes in apple fruits are compared with those of *G. album* and *G. fructigenum*. *G. perennans* grows at approximately the same rate as *G. album* and much more slowly than *G. fructigenum*.

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Explanation of Plate II

Fig. 1. Perennial Canker on Bramley's Seedling.

Fig. 2. Lenticel infection by spore suspension.
Left: *G. perennans*. Right: *Nectria galligena*. Centre: Technique used.

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STUDIES ON SOME DISEASES OF SAINFOIN (*ONOBRYCHIS SATIVA*)

I. RING-SPOT CAUSED BY *PLEOSPORA HERBARUM* (PERS.) RABENH.

By S. J. HUGHES, *University College, Cardiff*

(With Plate III and 3 Text-figures)

It appears from the literature that the diseases of sainfoin, or French grass as it is called in Glamorgan, have received very little attention in the past. Oudemans (1921) records seventeen different species and varieties of fungi as having been found on sainfoin in Europe and most of them are known only from the original diagnoses. Only three of these appear to have been recorded as causing diseases of sainfoin in Britain; the diseases are, Leaf Spot, caused by a fungus referred to *Ascochyta Orobi* Sacc.; Rust, *Uromyces Onobrychidis* (Desm.) Lév., and Mildew, *Erysiphe Polygoni* DC. (pro parte ≡ *E. Martii* Lév.).

Two diseases found in this country and caused by fungi not mentioned in Oudemans' list are: Chocolate Spot caused by *Botrytis cinerea* Fr. which has been observed in Glamorgan to kill flower buds and cause a stem rot under very moist conditions; and Rot caused by *Sclerotinia Trifoliorum* Erikss., first recorded on sainfoin in this country by Carruthers (1898), and reported several times since, by Carruthers (1902, 1907), Biffen (1910, 1912, 1913, 1918, 1920) and more recently by Rees (1936) and Moore (1944).

Grove (1935) found *Placosphaeria Onobrychidis* Sacc. (? ≡ *Diachora Onobrychidis* Müll. teste Grove) on *Lathyrus* at Kew, and not on sainfoin, the first recorded host for this fungus. Massee (1915) described by a brief translation of the account of Prillieux and Delacroix (1893) a leaf spot of sainfoin caused by *Ramularia Onobrychidis* Prill. & Delacr. This, however, is a doubtful British record and the fungus is not included in the List of Hyphomycetes recorded for Britain (Wakefield & Bisby, 1941). I found *R. Onobrychidis* on sainfoin in December, 1943.

This paper is the first of a series on diseases of sainfoin in Britain, particularly in Glamorgan. Sainfoin once played 'an important part in the agriculture of the Vale of Glamorgan especially on the thinner and drier Lias soils . . .' (Rees, 1936), and at one time it was grown on the Gower Peninsula. In 1944, the sainfoin growing area in this county comprised some seventy acres, at Gileston, Llantwit-Major and Southerndown, in the Vale of Glamorgan. It is also grown to a small extent in South Monmouthshire.

1. Ring-Spot, caused by *Pleospora herbarum* (Pers.) Rabenh. (Stat. conid., *Stemphylium botryosum* Wallroth).

This leaf Ring-Spot disease has not, as far as is known, hitherto been recorded in Britain; it was first found at Llantwit-Major and at the Cardiff University College Agricultural Experimental Plot at Ely in May 1943.

Frequent surveys have now shown that it is always present on sainfoin grown in Glamorgan. Ring-Spot, whilst present all the year round, is found in greatest abundance in the spring; it was very common at all stations in April 1944.

The ring-spot (Pl. III) typically shows a circular light brown area with a darker and well-defined margin. Young lesions are entirely dark brown but the central zone becomes lighter as the infection spreads. Under favourable conditions of moisture the fungus produces conidia abundantly, giving the spot a very conspicuous sooty appearance.

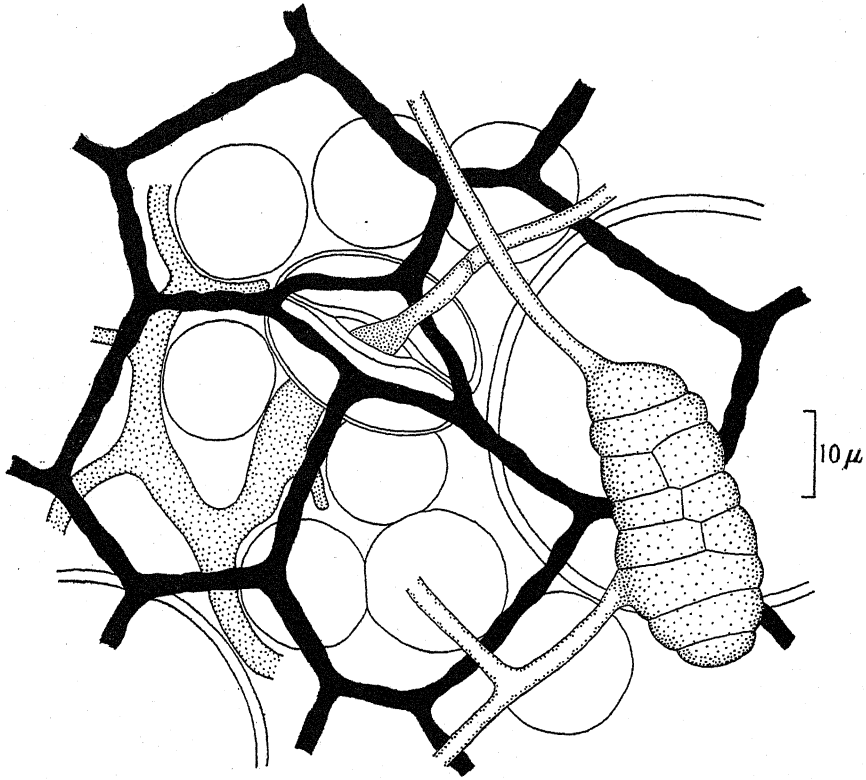
The fungus also flourishes on dead leaves and stems of sainfoin but no ring-spot effect is produced on them. It appears that the spread of hyphae into living tissues is essential for the production of the typical lesion.

The attacked leaflets usually wither and fall, thereby reducing leaf bulk, especially in the spring when the attack is at its height, but on the whole no appreciable damage has been caused to the Glamorgan crops. The ring-spots bear a resemblance to those caused by *Ascochyta Orobi* Sacc., and may well have been confused with them, but examination with a lens will reveal the immersed pycnidia of *Ascochyta* raising the epidermal layer of the diseased tissue. The conidial form of *Pleospora herbarum* has been found frequently in association with the *Ascochyta*.

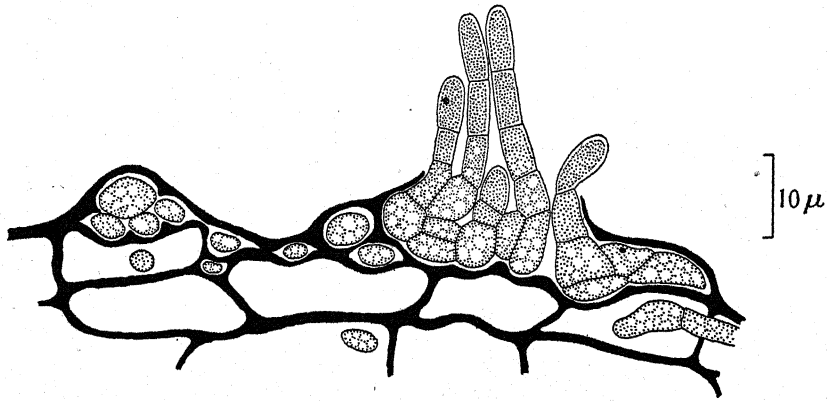
An aqueous suspension of conidia in an atomizer was used for spraying sainfoin leaves, and ascospores were introduced on to the leaflets as loopfuls of an ascospore suspension. In a very moist atmosphere under a bell-jar in the laboratory, sainfoin plants tend to shed their leaflets for some reason not connected with infection; to avoid this the atmosphere was kept only slightly moist to prevent dropping of the leaflets and to permit infection to take place. After two days the leaflets were removed and boiled in an alcohol acetic acid mixture, stained whole in cotton blue in lactophenol, and mounted in lactophenol as recommended by Smith (1940).

Fungal hyphae from both ascospores (Text-fig. 1) and conidia were seen to have entered the leaf through the stomata but not directly through the cuticle. Cuticular penetration may well take place but it is more difficult to observe. The penetrating hypha, once inside the substomatal cavity, widens considerably and eventually branches and ramifies intracellularly through the leaf tissue. This is almost identical with the description by Smith (1940) of the infection of lucerne by *Pleospora herbarum*, but he apparently observed cuticular penetration of the epidermis also. Fungal hyphae penetrate the epidermal cells from below and finally make their way between epidermal and cuticular layers. Proliferation occurs and subcuticular stromatic cushions are formed (Text-fig. 2). Further proliferation may result in the dissolution or rupture of the outer epidermal wall, with the formation of a deeper and more extensive cushion. The cuticle is torn open and the fungal cells push out hyphae which become thicker and darker walled than the deeper cells, and develop into a tuft of conidiophores. Subsequent development of the conidia has been fully described by Wiltshire (1938). The fungus fruits on both surfaces of the leaflet but more abundantly on the adaxial side.

From the form of the conidiophore and conidial characters and measure-

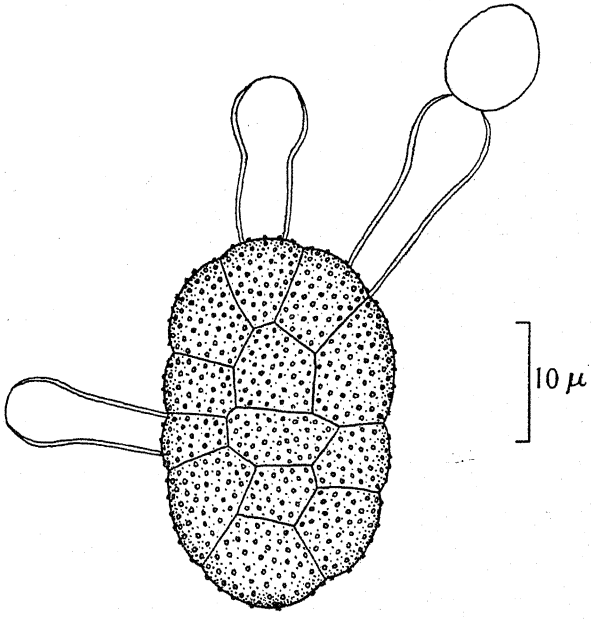


Text-fig. 1. *Pleospora herbarum.* Ascospore germinating on sainfoin leaflet with germ tube penetrating the stomate and swelling in the substomatal cavity. (In the preparation from which the drawing was made, by camera lucida, the penetrating hyphae originated from the ascospore, but for compactness of the figure they are shown unconnected.)



Text-fig. 2. *Pleospora herbarum.* Sectioned leaflet showing production of conidiophores from subcuticular cushion of fungal tissue.

ments, the fungus is referable to *Stemphylium botryosum* Wallroth. The conidial measurements differed somewhat from those recorded by Wiltshire for Wallroth's type specimen. He stated, however, that variation 'may be expected to occur on different hosts in nature and minor differences in spore size alone cannot therefore be regarded as satisfactory ground for maintaining species'. Again, Wiltshire stated that 'confirmatory evidence of the correct identification of the conidial stage is frequently afforded by the development of the ascigerous stage (*P. herbarum*)'.



Text-fig. 3. *Pleospora herbarum*. A conidium producing conidiophores directly.

Portions of cultures from single conidia were planted on rabbit-dung agar with straw added. When these cultures were placed in the light at a temperature of 10–12° C., as advised by Smith (1940), perithecia were produced in abundance. Asci with mature ascospores were formed in about three weeks. It is interesting to note that perithecia of *P. herbarum* were found on dead sainfoin stems in 1942 before any leaf-spotting was observed. Ascospore measurements, obtained from these perithecia, and conidial measurements are given below:

	Substrate	Range	Mean of 25
Ascospores	Dead sainfoin stems	32·4–36·0 × 15·3–17·1 μ	34 × 16 μ
	Dung-agar and straw	31·5–38·7 × 13·5–18·0 μ	35 × 16 μ
Conidia	Dead sainfoin stems	28·8–46·8 × 13·5–20·7 μ	36 × 17 μ
	Leaf-spot on sainfoin	23·4–39·6 × 14·4–21·6 μ	32 × 17 μ

During germination-tests on unmilled sainfoin 'seed', *Stemphylium botryosum* has been frequently met with, growing presumably sapro-

phytically on the pods. Perithecial fundaments developed but did not produce ascospores under the conditions of the test. It is interesting, in view of these facts, to record the finding of a browning and drying-out of one or both cotyledons in a few seedlings from such a sample of seed. *Pleospora herbarum* has been isolated from the diseased tissues, and in a few instances *Botrytis cinerea* Fr.

In Text-fig. 3 a single conidium is shown producing typical conidiophores with a spore initial at the apex of one; this has apparently been noted by the brothers Tulasne (1863) who stated 'we even think we have observed the sarciniform germs produce conidia of the same kind'.

I wish to thank Mr J. Rees for suggesting to me the need of a study of sainfoin diseases. I am also indebted to Mr W. C. Moore for reading the manuscript and for helpful criticisms, and to Miss K. Pears for the photographs.

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EXPLANATION OF PLATE III

A. Infected leaves of sainfoin plants (P_6, Glam. strain) from Experimental Plot, July 1943. The ring-spots are clearly seen.

B. Infected leaves of commercial Hampshire strain of sainfoin from Gileston, March 1944. All stages of infection are shown, from small brown spots to ring-spots. The sooty appearance of some leaflets is evident.

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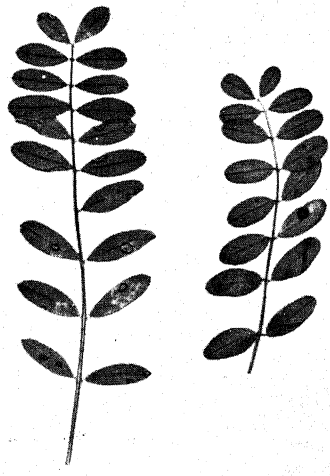


A

A LEAF SPOT OF MANGOLDS CAUSED BY *PLEOSPORA HERBARUM* (PERS.) RABENH.

By S. J. HUGHES, *University College, Cardiff*

(With Plate IV)

A spotting of mangold leaves was observed in October 1943 at Llanvaches, Monmouthshire, where the outbreak was reported to have appeared suddenly after heavy rain and general moist conditions.

The spots were circular, 1 mm. to about a centimetre or more in diameter, and on badly infected leaves they coalesced and caused extensive discoloration. The older spots showed concentric rings of light and dark brown regions with well-defined outer margins. Even in the field the fungus produced abundant conidia, giving the spots a sooty appearance. Only *Stemphylium botryosum* Wallroth (*Pleospora herbarum*) was seen on the spots. Leaves at various stages of infection are shown in Pl. IV.

Potash deficiency symptoms were also evident throughout the crop. Some plants were more badly affected than others and showed premature withering of the older leaves, which drooped around the crown like a rosette. The yield of such plants did not appear to be adversely affected as the roots had swollen well, though some had hollow centres; the total yield was estimated at forty tons to the acre. The leaf-spotting developed after the roots were fully grown and would not, therefore, be expected to have reduced the bulk of root. An analysis carried out by Dr W. T. H. Williamson, the Adviser in Agricultural Chemistry, showed that the soil was deficient in potash (exchangeable $K_2O = 0{\cdot}006\%$). This is not unexpected because there had been excessive use of nitrogenous manures, including dung, but only one application of potash, with potatoes, turnips with kale, and mangolds as successive crops.

Whilst most plants showed some leaf-spotting due to *Pleospora herbarum*, the plants suffering from potash deficiency were by far the most badly attacked. The lesions were more numerous and extensive on both green and partly withered leaves, although the innermost and younger unfolding leaves were usually not attacked. These facts suggest that the spot is largely associated with a weakening of the older leaves and also with the deficiency of potash in conjunction with the high nitrogen status.

The fungus obtained from the leaves was *Stemphylium botryosum* Wallroth. Its perfect stage, *Pleospora herbarum*, was obtained in culture on dung-agar and straw, in a good light and at a temperature of 10–12° C. Conidial and ascospore measurements are given below:

	Range	Mean of 25
Ascospores	34·2–41·4 × 14·4–18·0 μ	37 × 16 μ
Conidia	29·7–42·3 × 14·4–23·4 μ	34 × 19 μ

Inoculation experiments were carried out on small, apparently healthy plants taken from the infected field, potted up in soil, and kept indoors under a bell-jar in a moist atmosphere.

Preliminary experiments, using conidia and portions of agar media bearing mycelium, showed no infection if the leaf were undamaged. Ascospores were not available in sufficient numbers to carry out similar inoculations.

Further experiments with conidia and mycelium, on leaves damaged locally by scratching with a needle, showed that infection took place readily, and typical ring-spot lesions were produced. Sections of the diseased leaves showed hyphae of the fungus inter- and intracellularly. *Pleospora herbarum* was re-isolated from the diseased tissues at some distance from the point of inoculation, after sterilizing the surface of the leaves with mercuric chloride solution. When treated similarly, inoculated petioles, previously damaged, were also attacked but the roots remained unharmed.

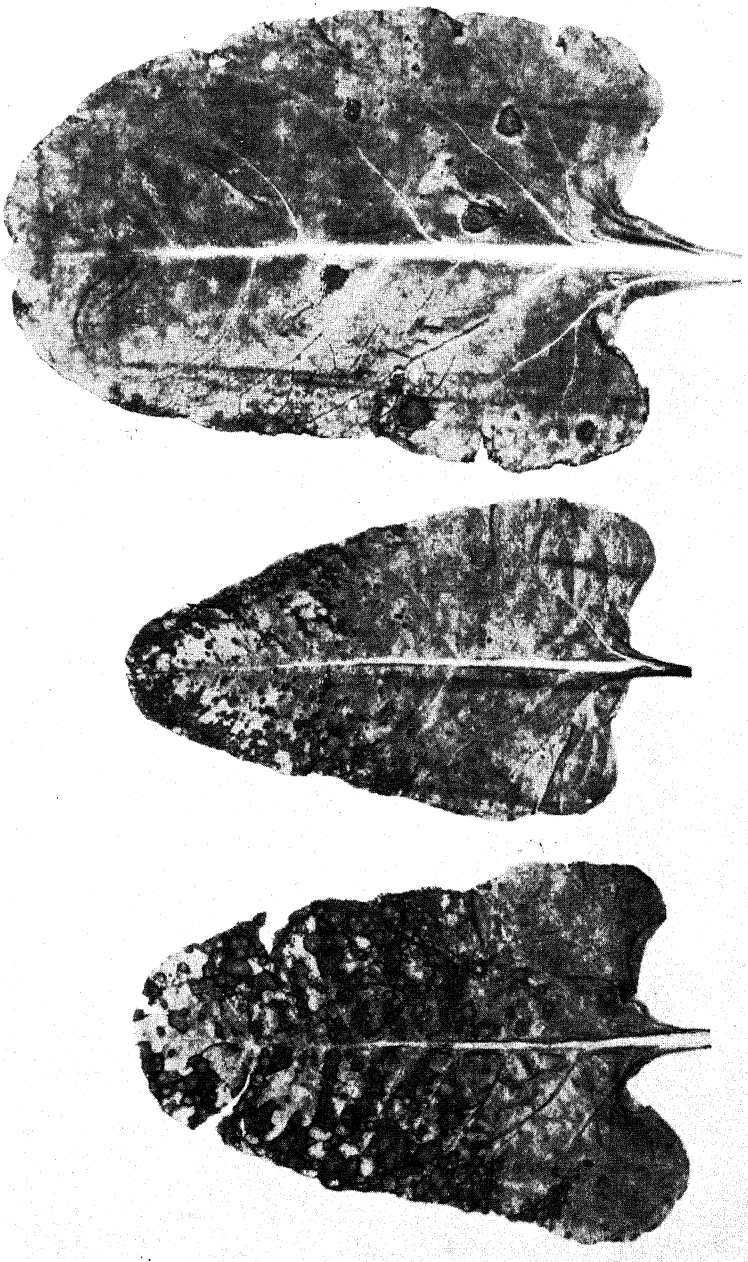
The fungus *Pleospora herbarum* and its conidial stage *Stemphylium botryosum* have recently been reviewed by Wiltshire (1938). A search through the *Review of Applied Mycology* has revealed that the fungus has a very wide host range; it has been recorded very frequently as the cause of a black mould on onions. Edgerton (1921) concluded that the fungus develops rapidly on any slightly weakened onion tissue, whether mechanically injured or not. Both Edgerton (1921) and Machacek (1929) stressed that great humidity predisposes onions to attack by *P. herbarum*. Such moist conditions prevailed when the mangold leaves were attacked.

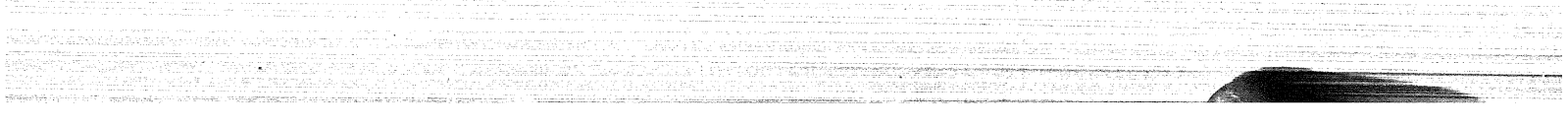
Teodoro y Gregorio (1923) recorded positive results after inoculating both injured and non-injured onion leaves but he did not state whether or not they were healthy. On the other hand, *P. herbarum* is recorded by other workers merely as an associate of Downy Mildew (*Peronospora destructor* (Berk.) Casp.); thus, on garlic by Caballero (1922) and on onions by Bremer and Nicolaisen (1934). It is also stated (Cotton, 1922) that *Macrosporium parasiticum* Thüm. (*Pleospora herbarum*) is 'An almost invariable accompaniment of Mildew, and apparently increases the damage caused by *Peronospora*'. There does, indeed, appear to be a measure of doubt as to the extent of the parasitic nature of *P. herbarum* on onions.

Further recorded hosts include lettuce (Ogilvie & Mulligan, 1931; Dippenaar, 1939), *Arctostaphylos manzanita* (Briant & Martyn, 1929), bean seeds (Brinkman, 1931), beetroot (Neuwirth, 1925), apples (Carter, 1935), Clarkia (Lewis, 1937), tomato fruits (Small, 1936), lucerne (Smith, 1940), endive (Moore, 1943) and sainfoin (Hughes, 1945).

Serbinoff (1927), after careful observations in the field and laboratory, concluded that, near Odessa, diseases of cultivated plants caused by species of *Macrosporium* and *Alternaria*, including *M. parasiticum* (*P. herbarum*), are secondary only to attack by different species of bacteria. He regarded the fungus under discussion as either a facultative or a weak parasite.

This paper adds another host to the list of plants capable of being infected by *P. herbarum*. Here, however, the leaf-spotting was probably promoted by a weakening of plant resistance brought on by potash deficiency in conjunction with other unbalanced soil conditions. The *p*H





of the soil was 5·4, which is considered too low for mangold cultivation, but whether or not this affects the resistance of the mangold is not known.

I wish to thank Mr J. Rees, under whose direction the work was carried out, for bringing the disease to my notice. I am indebted to Mr W. C. Moore for reading the manuscript and for helpful suggestions, and to Miss K. Pears for the photographs.

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EXPLANATION OF PLATE IV

Mangold leaves at various stages of infection.

(*Accepted for publication 24 January* 1945)

THE TRUE NATURE OF *MYRIOBLEPHARIS* THAXTER

By GRACE M. WATERHOUSE, *Lincoln Training College*

(With Plate V and 4 Text-figures)

When *Myrioblepharis* was found in the Cropston stream, Leicester, by Dr C. T. Ingold and kindly handed over to the Royal Holloway College Botanical Department, it offered a good chance to settle the problem of this genus. The organism was first found there in June 1941 growing on the petioles of decaying alder (*Alnus glutinosa*) and other leaves which had fallen into the stream. Samples have been taken each summer since 1941, and although detailed observations of the time of appearance have not been made, it seems that *Myrioblepharis* usually appears towards the end of June and disappears during late August. A further record of its occurrence was obtained by one of Dr Ingold's students (B. Plunkett), from Evington Brook, south-east of Leicester, in 1943 as late as September. These are the first records of *Myrioblepharis* in the British Isles.

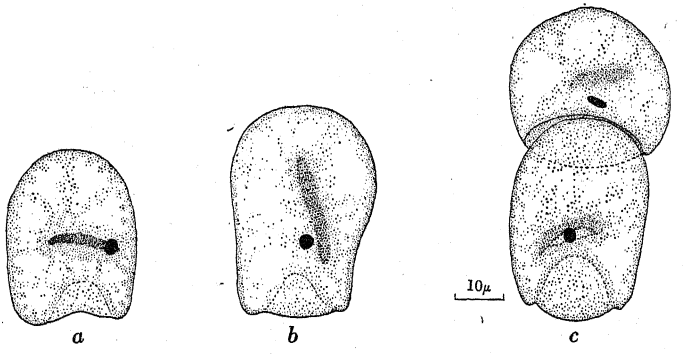
The leaves, at a stage when they were quite brown but not very decayed, were collected from the stream, the laminae were cut off and the petioles washed in tap water and left in open glass dishes in tap water. If examined immediately, specimens of *Myrioblepharis* could usually be seen on a few petioles. Numbers increased considerably in a day or two if left in water (refreshed at intervals).

The status of *M. paradoxa* rests on two descriptions, those of Thaxter and von Minden. The original one by Thaxter (1895) interpreted *Myrioblepharis* as a peculiar genus of aquatic Phycomycetes characterized by multiciliate zoospores but in other respects resembling proliferating species of *Pythium*. Von Minden (1915), however, concluded from his, unfortunately incomplete, observations that there were two organisms present, (1) a *Pythium* of the *P. proliferum* type and (2) a parasitic animal organism —a ciliated protozoan—preying on the sporangia of the fungus. The present investigations which were made in the Royal Holloway College Laboratory in July 1941 and 1942 and at University College, Leicester, in July 1943, establish that von Minden's interpretation was correct: he would doubtless have proved this fully had he been able to complete his observations.

When the petioles collected from the stream were examined, various Protozoa could usually be seen browsing in the tangled web of fungal hyphae, algal filaments and bacteria clothing the surface. One species, rather larger than most, and of a barrel shape, moved about with a slow rolling motion, and it could be seen settling down on the coenocytic hyphae projecting from the substratum. It was this protozoan which proved to be the animal partner of the *Myrioblepharis* association. It was oval or pyriform with a terminal mouth, ciliated all over, and it swam, with the

mouth directed forwards, in a spiral path, rotating about its long axis. It had a single laterally placed contractile vacuole which burst every 6–12 sec. The protoplasm was rather densely granular except in the mouth region where it was clear. Staining showed clearly two nuclei, a large irregular meganucleus and a smaller spherical micronucleus (Text-fig. 1). This feature and the covering of cilia characterize the organism as a member of the class Ciliata of the Protozoa. It has been identified provisionally by Mr A. G. Willis of the Zoology Department of Manchester University as probably a species of *Prorodon*.

The coenocytic hyphae projecting from the petioles were unbranched and grew either in tufts or singly. If unattacked by the ciliate the tufted ones, which were stouter, produced sporangia and zoospores characteristic of proliferating species of *Phytophthora*. The more slender single hyphae produced sporangia and zoospores as in proliferating species of *Pythium*. These were the fungi that the animal used as its hosts. The most detailed

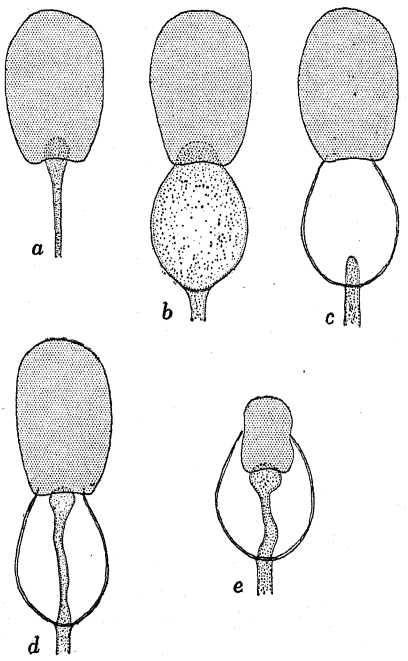


Text-fig. 1. The ciliate showing the meganucleus, micronucleus and mouth; (*a*) young ciliate; (*b*) mature ciliate; (*c*) after the first division. Stained haematoxylin, drawn camera lucida.

observations were made when the ciliate was using the species of *Phytophthora* as host. In the following account therefore, this is the fungus described and figured (Pl. V, figs. 1, 2), but the process when a species of *Pythium* is used as host is essentially the same.

The ciliate always settled down with its mouth over the projecting parts of the fungus (Text-figs. 2, 4), sometimes over a hyphal tip swelling to form a sporangium (Text-fig. 2 *a*, *d*), or over the papilla of a mature sporangium (Text-fig. 2 *b*), or over the opening of an empty sporangium (Text-fig. 2 *c* and Pl. V, figs. 3, 5). Occasionally a young ciliate would squeeze partly into the sporangial opening so that its mouth was over the tip of the hypha proliferating from the base (Text-fig. 2 *e*). As the hypha grew it pushed the ciliate out of and away from the orifice. If the sporangium was nearly mature when the ciliate settled on it, then it dehisced normally, most of the zoospores escaping as the ciliate was pushed away by the swelling vesicle. Some might be trapped in the sporangium as the ciliate settled down on it again. The zoospores were not consumed by the ciliate, indeed, one rounded off almost inside its mouth and could still be seen

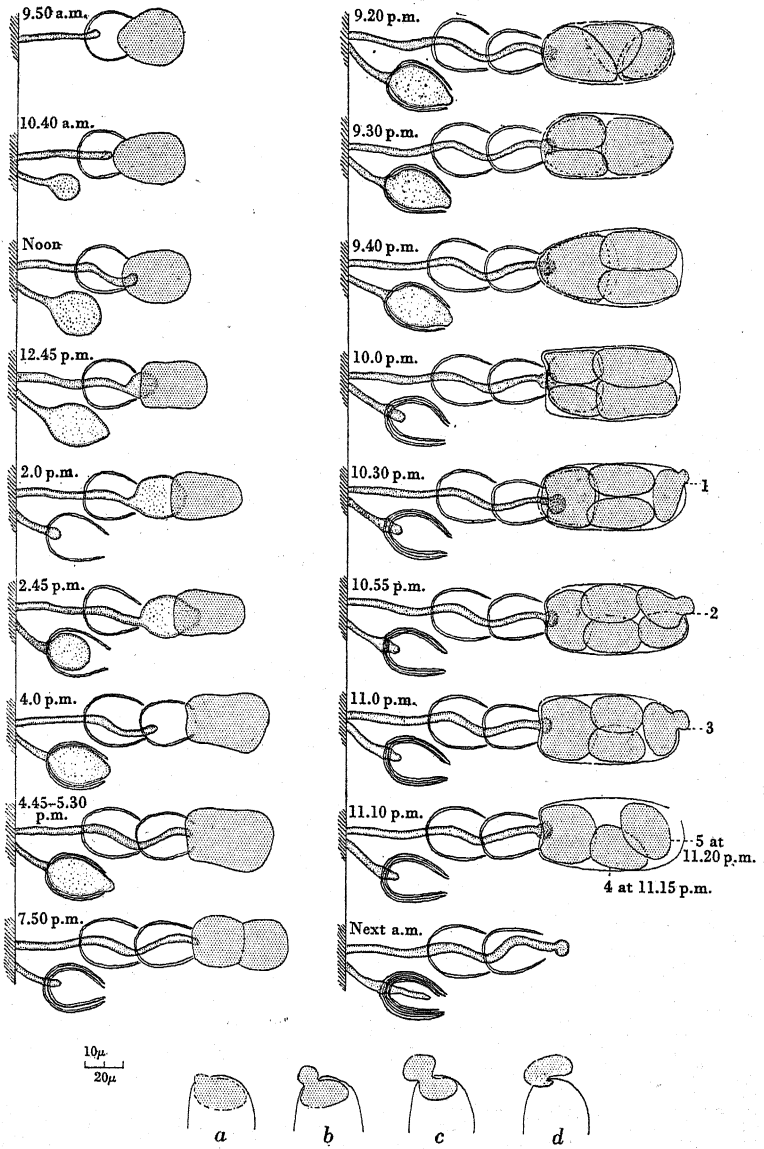
there later. Zoospores were not formed, however, if the ciliate had been in contact with the fungus for any length of time; instead, the contents of the sporangium came out as an undifferentiated liquid mass. This was often seen when the ciliate had been disturbed and had left the sporangium shortly before dehiscence. What happens to the contents when the ciliate remains *in situ* it is not possible yet to say, as a sporangium has not been caught in the act of dehiscence. It is presumed that some or all of the contents pass into the mouth of the ciliate, because sporangia observed



Text-fig. 2. Diagram showing how the ciliate settles mouth downwards on the fungus in different stages of development; (*a*) over the tip of a young hypha swelling to form a sporangium; (*b*) over a mature sporangium; (*c*) over the mouth of an empty sporangium; (*d*) over the tip of a hypha proliferated from the base of an empty sporangium; (*e*) young ciliate squeezed into the opening of an empty sporangium to get its mouth over the swollen hyphal tip. No special magnification. Protoplasm represented diagrammatically by dark stippling in the ciliate and by paler stippling in the fungus.

shortly after emission leave no trace of the contents in the neighbourhood of the opening.

When the organism had settled down on the fungus it became more cylindrical (Text-fig. 4). It rotated slowly; more quickly when fresh water was added. The contractile vacuole could be seen enlarging and suddenly disappearing. Gradually the ciliate grew, and when it reached a certain size (usually after emission of the contents of one sporangium) division took place. A median constriction appeared between the mouth and the posterior end (Text-fig. 4 *b*), indicating that it was about to divide. A young specimen which was watched (in water in an open dish under

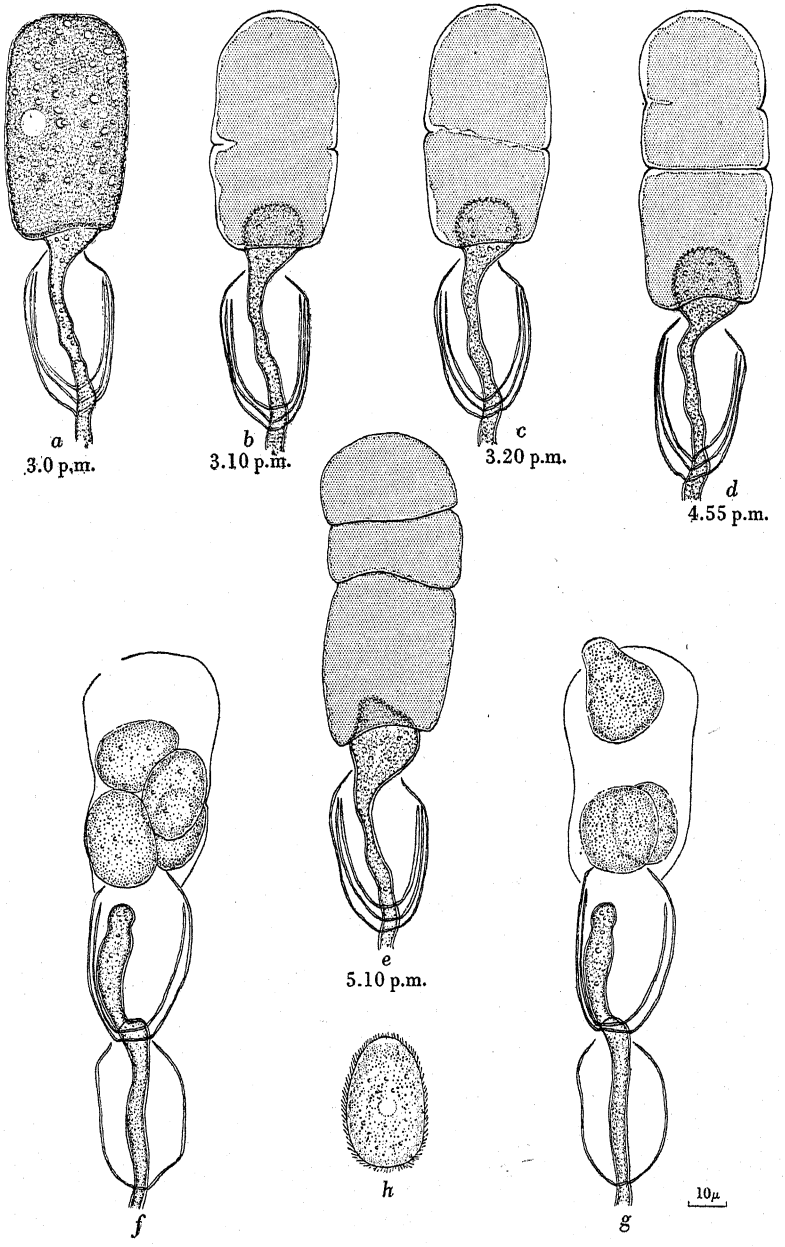


Text-fig. 3. Growth and division of the ciliate and escape of the daughter ciliates produced from the division; 9.50 a.m.–4.45 p.m. growth of young ciliate; 7.50–10.20 p.m. divisions of ciliate; 10.30–11.10 p.m. escape of the products (order of escape 1–5). An uninfected fungus sporangiophore grew up at the side and produced three successive sporangia which dehisced normally. *a–d*, escape of first daughter ciliate through pore in cyst. Drawings camera lucida. Protoplasm shown diagrammatically as in Text-fig. 2. Substratum shown diagrammatically.

the microscope) during its development took about eight hours to reach this stage of division (Text-fig. 3). In the course of the next hour cleavage into two was completed. Cleavage began with a dip in the cuticle, and a few minutes later cleavage of the outer region of the protoplasm could be seen. This proceeded inwards until the protoplasm was cut in two (Text-fig. 4 *c*). In one specimen this division took twenty minutes. During division the contractile vacuole was not seen and rotation usually ceased. Then followed a quiescent period of an hour or more during which the organism (both halves) grew. A second cleavage, similar to the first, then took place but only in the posterior half (Text-fig. 4 *d*, *e*). Rotation sometimes began again at this stage, each part moving independently. The two posterior ones or even all three might swim away, especially if disturbed, but usually further divisions followed (Text-fig. 3). These divisions were difficult to see owing to the density of the protoplasm and movement of the products. It was not possible to see in what plane the final divisions occurred, but it seems probable from knowledge of division in ciliates generally that the subsequent cleavage planes were parallel to the first. The four products of the third division became so disposed that two lay posterior to the other two. Sometimes five daughter ciliates were ultimately produced from the upper half. How the division proceeded to produce the fifth could not be seen. When the organism was fully divided the products lay within a pellicle or cyst and moved there for some time before they escaped (Text-fig. 4 *f*, *g*). A pore was formed in the cyst and the daughters squeezed through one by one. The young ciliate thus produced was about 9–12 μ long and pyriform, the narrower anterior end being clearer than the rest of the protoplasm (Text-fig. 4 *h*). It swam for a short time only before settling down on the fungus and restarting the cycle. The anterior half of the organism which remained in contact with the fungus sometimes swam away as well or else it remained to grow and divide again or sometimes it seemed that it divided at the same time as the posterior half.

The sequence of events was thus very much as Thaxter described it. The most conspicuous difference was in the speed of the changes taking place. The time taken for the fully grown organism to segment and discharge its products was only half an hour in the North American material, whereas in the English material it was six hours. Thaxter commented on the speed and violence of the rotating movement. It was evidently the rapidity of growth and movement which obscured the details and led Thaxter to interpret the products of division as the zoospores of the fungus. He did at first consider that he had an epizooic or ectoparasitic creature but later discarded the idea.

This account is given to show that *Myrioblepharis* is really two organisms, viz. a fungus and a protozoan. It does not elucidate the relationships between the two, as more observations will be necessary before this can be done. Is the animal using the fungus merely as a convenient perch while it divides, or is it absorbing nutriment from the fungus? Facts which point to the latter view are (1) that the ciliate settles with its mouth over the hyphal tip, (2) it grows while it is situated there, (3) the contents of



Text-fig. 4. *a–e*, stages in the division of the ciliate; (*a*) mature ciliate with its mouth over a developing sporangium of the fungus, revolving slowly, contractile vacuole showing; (*b*) first sign of cleavage in ciliate, movement has ceased, no contractile vacuole evident; (*c*) first cleavage complete; (*d*) beginning of second cleavage; (*e*) second cleavage complete; movement then began again and all three parts swam away 5.25 p.m. *f–g*, escape of daughter ciliates; (*f*) one daughter has already escaped through a pore in the cyst; (*g*) young ciliate escaping through pore; (*h*) young ciliate after escaping. Camera lucida. Protoplasm shown diagrammatically by stippling in *h*.

the sporangium, although not seen to pass into the animal, were not to be found in the vicinity soon after emission (von Minden stated that the entire contents were emptied into the revolving body), (4) after the ciliate has rested on the sporangium for a time it fails to produce zoospores but instead gives out a mass of protoplasm: this suggests that the ciliate is affecting the fungus adversely although not necessarily absorbing substance from it, (5) the newly formed ciliate has a very short swimming phase before it settles down on the fungus.

The species of both *Phytophthora* and *Pythium* used as hosts have been grown in culture but have not yet been identified. One would like to isolate the ciliate in culture too and then reunite the partners to produce the *Myrioblepharis*. It is probable that so specialized a ciliate might not live in culture. The cultured fungus, however, might be reinoculated with the ciliate from the stream at the appropriate time of year. Even without these final proofs it can be said definitely that *Myrioblepharis* as a genus is no longer valid, although one might refer to the *Myrioblepharis* association which occurs between a ciliate and some aquatic species of *Pythium* and *Phytophthora* at a certain time of the year.

Summary

Evidence is brought together whereby the long-suspected double nature of *Myrioblepharis paradoxa* Thaxter is finally demonstrated. What may now be termed the *Myrioblepharis* association is provided by a ciliate and a species of either *Pythium* or *Phytophthora*.

I acknowledge with gratitude the help given by Miss E. M. Blackwell and by Professor C. T. Ingold, particularly for the photographs which he took and which are used for Plate V.

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Explanation of Plate V

Figs. 1, 2. Typical sporangia of the species of *Phytophthora* used as host.

Fig. 3. Mature ciliate over the mouth of an empty sporangium, the fungus hypha is proliferating from below and swelling to form another sporangium. The ciliate shows signs of division.

Fig. 4. First division of the ciliate in process while situated over a mature sporangium. To the right a young ciliate on a sporangium.

Fig. 5. Ciliate fully divided into three, lower half with a slight constriction prior to division; situated over an empty sporangium with the proliferating hypha grown up into the mouth of the ciliate.

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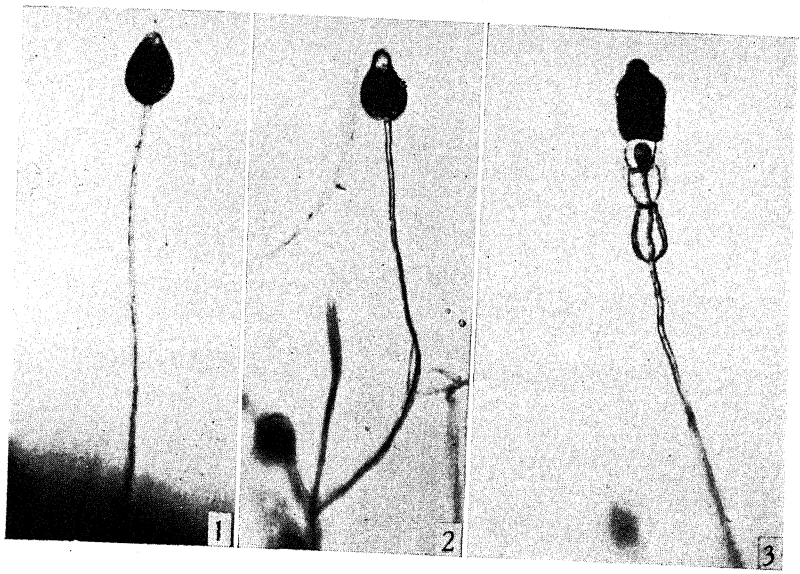
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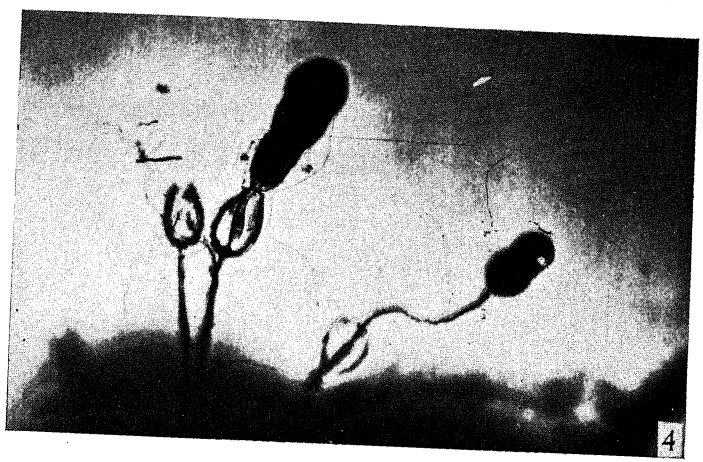
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5

STILBUM TOMENTOSUM SCHRAD.

By T. PETCH

Historical

This fungus, a common parasite of *Trichia* and other Myxomycetes, was described by Schrader (1799) as '*Stilbum tomentosum*, stipite tomentoso, capitulo subrotundo'. Schrader stated that it grew on *Trichia* and other allied species, and also on sheep dung, first appearing as a small white tomentose patch, from which there soon arose a slender stalk, clothed with similar tomentum, the specific epithet being chosen because of the tomentum on the stalk. It is to be noted that, as Schrader stated that it grew on *Trichia* and on sheep dung, he evidently confused two different fungi. His enlarged figure of a specimen on a *Trichia* shows a glabrous stalk.

Persoon (1801) quoted Schrader's name and description, with the alternative name, *parasiticum*, and suggested that the tomentum was some *Byssus* which had developed on the stalk during drying, and hence the name *parasiticum* would be more suitable.

Albertini and Schweinitz (1805) recorded *Stilbum tomentosum* Schrad. on *Trichia*, *Cribraria vulgaris*, *C. cernua*, etc., and noted that in their specimens both the tomentum on the stalk and the subiculum on the host were always present.

Ditmar (1817) adopted the name *Stilbum parasiticum* Persoon. He stated that the stalk was glabrous, and that the hyphae observed by Schrader, which occurred sometimes, but not always, at the base of the stalk, appeared to him to be an intrusive mould, as its varying occurrence would seem to indicate. He gave figures of the fungus growing on '*Trichia nitens* Persoon', and his enlarged figure shows the stalk minutely rough.

Persoon (1822) listed it as '*Stilbum parasiticum*, stipite glabro, tomentulo insidente', adding that the stalk was glabrous but arose from a byssoid subiculum, and noting that Albertini and Schweinitz had recorded that they had always found the stalk tomentose. He did not, as has been stated, distinguish two forms, but merely adopted his previous alternative name.

Greville (1827) described the stalk of *S. tomentosum* as glandulose, set with minute pellucid rounded processes or glands, and the conidia as very minute, globose. He added: 'It is true, that in some specimens a fine delicate mucor-like substance is said to envelope the whole plant; but that is an extraneous appendage. In its natural state, it is completely destitute of any such appearance.' Greville (1823) had previously described, on *Trichia*, *Isaria microscopica*, a very minute species, in which he was unable to find conidia. Fries (1829) suggested that the latter was immature *Stilbum tomentosum*, and that view has been generally accepted.

Fries (1829) adopted Schrader's name, describing the fungus as having a tomentose stalk united to a byssoid mycelium, with a form *b*, stalk yellowish, somewhat glabrous, which he suggested was an older state. For

the type he cited Schrader (1799), Persoon (1801), Albertini and Schweinitz (1805), and Greville (1827), and for form *b*, Ditmar (1817) and Persoon (1822).

Berkeley (1836) gave to *Stilbum tomentosum* the English name, glandular *Stilbum*, and described the stalk as glanduloso-tomentose, recording it on *Trichia fallax* and *T. chrysosperma*.

Saccardo (1886) cited Schrader, Greville and Ditmar for this species, and described the stalk as glanduloso-tomentose, arising from a byssoid base. He did not give any spore measurement.

Oudemans (1886) recorded *Stilbum parasiticum* Pers. on *Trichia chrysosperma*, and stated that the flocci on the stalk described by Schrader were completely lacking in his specimens. Later (1903) he declared that the woolly surface of the stalk, on which Schrader based the name of the species, was not due to glands, as many authors had supposed, but to hyphae of the central column which bend outwards and bear each an apical conidium. Apparently it did not occur to him that those hyphal tips might be the glands of previous authors. Oudemans gave the conidia as globose, hyaline, 1·2 μ diameter, but in view of his statement it is uncertain whether he measured the glands on the stalk or the conidia on the head.

Massee (1893) gave the conidia as globose, 2–3 μ diameter, while Bresadola (1903) stated that they were 4–5 × 2–2·5 μ. The question of the size of the spores was dealt with by Miss A. L. Smith (1903) who wrote as follows:

'My interest in the fungus was aroused by the difference in the form and size of the spores, which puzzled me in my attempts to classify various specimens. The stalk I have always found to accord with Greville's description, "set with minute pellucid roundish processes". The spores in Greville's and Ditmar's figures are small rounded bodies. Saccardo describes them as "globosis, exiguis", and Massee, in his *Fungus Flora*, adds the exact measurement as 2–3 μ diameter. Specimens sent to me from Hampshire and Devonshire tally with the above descriptions; the stalks are beset with processes, the spores are globose, though smaller somewhat. They are extremely minute. I received still another specimen from Egham, in Surrey, which had a similar stalk, but the spores are oval in form and measure up to 5 × 2 μ. On examining *Stilbum tomentosum* in the National Herbarium at South Kensington, I found a Ceylon specimen in the Broome collection, in which the spores have been drawn and measured by that careful worker; they are oval in form and 0·0002 in. long, or 5 μ. There are other specimens in the Broome herbarium collected in England, but the spores are not figured. The difference between the two kinds of *Stilbum* amounts almost to a specific distinction, but the plants are otherwise so much alike that it seems better to distinguish the second as a variety. The form with globose spores has priority, the other I propose to name var. *ovalisporum*.'

Examination of the specimens in Herb. Mus. Brit. shows that the data have been confused, probably in writing up the notes. The slide of the Hampshire specimen is marked by Miss Smith, 'elliptical spores', and has oval spores about 5 μ long; the *Stilbum* is on *Trichia varia*, and was gathered at Alresford, Hants, March 1902, by the Rev. W. Eyre: another head from

this collection gave conidia 3–4·5 × 1–1·5 μ. The slide of the Egham specimen is marked, 'on *Trichia* from Egham with round spores'; it is on *Trichia varia*, and the head of the *Stilbum* is covered with a mass of minute granules; it is mounted in glycerine. Another slide from a specimen from St Catherine's shows minute oval conidia with acute tips, 0·75–1 × 0·5 μ, or globose conidia, 0·5 μ.

The Ceylon specimen referred to by Miss Smith is on *Hemitrichia serpula*, Thwaites no. 130. There is another Ceylon specimen, Thwaites no. 83, in Herb. Mus. Brit., on which Broome has drawn the conidium as oval, 0·00015 in., that is, 3·75 μ, though in the *Fungi of Ceylon*, no. 1003, the measurement was given as 0·0003–0·0004 in., or 7·5–10 μ. Measurements on fresh specimens on *Trichia Botrytis* in Ceylon gave in one case 1·5–2 × 0·75 μ, and in another 1·5–3 × 0·75–1 μ. But on examining the part of Thwaites no. 83 which Thwaites retained in the Peradeniya herbarium, I found that it bore conidia which attained a length of 10 μ or more. These, however, were not the conidia of the *Stilbum*. The latter was in turn parasitized by a hyphomycete which consisted of a few hyaline septate hyphae, about 3 μ diameter, twining round the *Stilbum* stalk and head and producing solitary oval *Cephalosporium* heads, up to 13 × 7 μ, each containing up to eight conidia in a parallel bundle, the conidia being hyaline, cylindrical, 10–12 × 2 μ, with some only 4–5 μ long. That was tentatively referred (Petch, 1912) to *Cephalosporium stellatum* Harz (1871), recorded as parasitic on *Stilbum bulbosum* and *S. vulgare*, but when comparison with Harz's original figure and description was possible, it was seen that that identification was wrong, if Harz's figure is correct. That, however, is immaterial to the present discussion, the relevant point being that the long conidia observed on the Ceylon specimen, Thwaites no. 83, are those of a hyphomycete parasitic on *S. tomentosum*.

Berkeley and Broome (1873) found on *Hemitrichia serpula*, Thwaites no. 83, a perithecial stage which they described as *Hypomyces stilbiger*, and they added to their description the note, 'It is very interesting to ascertain that *Stilbum tomentosum* Schrad. is merely the conidiophore of a *Hypomyces* parasitic on *Trichiae*'. This species now stands as *Byssostilbe stilbigera* (B. & Br.) Petch (1912). I have collected it in Ceylon on *Trichia Botrytis* and *T. affinis*, and it has been recorded from Java as *Ophionectria* (*Ophiostilbe*) *Trichiae* Penz. & Sacc. I have not seen any indication of this species on any British specimen of *Stilbum tomentosum*, nor have I found any record of its occurrence elsewhere. Bommer and Rousseau (1884) gave *Hypomyces stilbiger* as the perithecial stage of *Stilbum tomentosum*, but they were merely quoting from Berkeley and Broome.

Stilbum tomentosum has been found in Ceylon on *Hemitrichia serpula*, *Trichia varia*, *T. affinis* and *T. Botrytis*, and is especially common at Hakgala on the last-named species. The stalks are always glandular, and usually arise from the sporangia without, or with only a slight, basal mycelium. Small white isolated patches of mycelium generally indicate perithecia.

Specimens of *Stilbum tomentosum* in herbaria are often unserviceable, as the head is easily broken off when dry. Conidia have been found on the following specimens in Herb. Kew.

'Queen's Cottage Grounds, Kew', n.d.; on *Trichia varia* and *T. affinis*; heads not found; specimen on *T. varia* bears loose conidia, cylindrical, ends rounded, 8–10 × 2–4 μ, and oval conidia, 4 × 1·5–2 μ, lying together.

'On *Trichia clavata*, Shere, October 1866, D. Capron'; is on *T. varia*; no conidiophores on stalk or base; conidia oval, 1·2–2 × 0·75–1 μ, a few globose, 1 μ.

'E. Vize, *Microfungi Britannici*, 354, Forden'; on *Trichia varia*; conidia oval, ends rounded or subacute, 4–6 × 2 μ.

'On *Trichia affinis*, Eastham Rake, 14 January 1911, Herb. Dr J. W. Ellis'; is on *T. varia*; no conidiophores on stalk or base; conidia in the head oval, 2–4 × 1–1·5 μ; a loose cluster of subcylindrical conidia, 8–10 × 2–2·5, and another group, subcylindrical, narrow oval or fusoid, 4–6 × 2 μ.

'Thwaites no. 83, Peradeniya [Ceylon], November 1867'; on *Hemitrichia serpula*; conidia oval, ends acute or obtuse, 2·5–4 × 1–1·75 μ. It is to be noted that *Hypomyces stilbiger*, which is also Thwaites no. 83, was collected in December 1868.

'On *Trichia Botrytis*, Hakgala [Ceylon], May 1910, no. 257'; conidia in the heads rod-shaped, ends rounded, 3–4 × 0·5 μ; subcylindric conidia 9–12 × 1·5–2 μ also present.

The differences thus far revealed in the published descriptions and the herbarium specimens relate to the character of the stalk and the dimensions of the conidia. But Grimm (1889–90) had raised another question. He had found *Stilbum tomentosum* common in the neighbourhood of St Petersburg, and had grown it in culture. He stated that, about a month after sowing the conidia, small cushions appeared, consisting of a mass of united vertical hyphae, which produced branches with terminal conidial *Cephalosporium* heads over the whole surface of the cushion. This structure increased in height, and then the hyphae branched at the apex, the branches producing similar conidial heads which fused into a globose terminal head. Grimm figured the fungus on *Cribraria argillacea*, *Dictydium umbilicatum* and *Arcyria cinerea*, and stated that the conidia were 9 × 4 μ. His figure shows an isarioid structure with suberect *Cephalosporium* conidiophores, each with its separate head of conidia, diverging from it, and a large globose terminal head of conidia.

Apparently, Grimm's paper passed unnoticed until it was taken up by Lindau (1909), who, on the facts given therein, transferred *Stilbum tomentosum* to *Tilachlidium*, as *T. tomentosum* (Schrad.) Lind. Lindau gave the conidia as cylindrical, obtuse, 3·5–7·5 μ long, 2–2·5 μ broad, rarely almost globose, 3·5 μ diameter, though he quoted Grimm's measurement, 9 × 4 μ, in his summary of the latter's paper. He added that while Oudemans found, laterally on the stalk, only conidiophores with a single apical conidium, Grimm under more favourable conditions found conidiophores with complete *Cephalosporium* heads. He did not recognize that Oudemans' conidia on the stalk were probably the glands, and that Grimm's conidia are something quite different. What had become of the glands in the structure Grimm obtained is not recorded. Meanwhile it may be said that nothing similar to Grimm's fungus has been observed to occur on Myxomycetes in nature, and it is doubtful whether it should be regarded as a

Tilachlidium. The type of the latter genus is a solid, simple or branched clava, with *Cephalosporium* conidiophores more or less perpendicular to the stem, not an isarioid group of diverging conidiophores.

Ferraris (1909, 1910) objected to Lindau's transference of this species to *Tilachlidium*, and stated that after examining numerous examples he had not been able to find the lateral heads described and figured by Grimm. He gave a figure of the stalk with its minute projecting hyphal ends, the glands, and described the conidia as typically very minute, obovate, $1{\cdot}2 \times 0{\cdot}5\mu$, or subglobose, $1{\cdot}2\mu$ diameter, or in the variety *macrospora* Ferr., founded to distinguish the form recorded by Bresadola, $3–5 \times 2–2{\cdot}5\mu$. The latter is apparently var. *ovalisporum* A. L. S.

Von Höhnel (1916) transferred *Stilbum tomentosum* to *Dendrostilbella*.

Recent observations

An extensive growth of *Stilbum tomentosum* on *Trichia varia* and *T. persimilis* on the under-side of a fallen trunk on swampy ground was found in Holt House Wood, near King's Lynn, 3 April 1930. The *Stilbum* fructifications were up to 1·75 mm. high, with a stalk up to 0·25 mm. diameter, and a head up to 0·36 mm. diameter, or larger by fusion with adjacent synnemata. In some instances the stalks were branched, a decumbent stalk giving rise to erect lateral branches of the same character as the parent.

The stalk is invariably composed of parallel agglutinated hyphae, and sparsely or densely covered with minute spore-like bodies. Some of the latter are globose, up to 4μ diameter, and these are usually lateral and sessile on the exterior hyphae of the stalk; others are oval, up to $8 \times 4\mu$, and these are either lateral, like the globose bodies, or are the projecting tips of the stalk hyphae. All these bodies are hyaline and minutely verrucose, and in some specimens the exterior hyphae of the stalk are also minutely verrucose. These spore-like bodies are no doubt the 'glands' of Greville, and probably the one-spored conidiophores of Oudemans; they are typical of *S. tomentosum* and are figured by Ferraris, but except for some of the oval bodies there are no free hyphal ends and the stalks are not tomentose in the usual sense.

The young synnemata, before the formation of the head, have, as a rule, no white basal patch, and the smaller mature synnemata either lack a basal patch or have a small repent fleck of mycelium at the base which is scarcely discernible until the specimen is mounted. The larger and older specimens in this collection had a visible white basal patch. The repent hyphae of this patch bear lateral sessile spore-like bodies, like those of the stalk.

The conidiophores in the head are narrow flask-shaped or conical, $12–16\mu$ long, $1{\cdot}5\mu$ diameter below. In very small heads they arise from the apices of the fused hyphae of the stalk, but in the larger heads the stalk hyphae separate above and the conidiophores arise in clusters of up to four from the apex of a stalk hypha, with sometimes a single conidiophore from the first node below. In general, the flask-shaped conidiophore has a short neck, about 3μ long, on which the conidium (oval in these specimens) is

sessile, but in some instances the thin apex is produced to double that length and terminates in a globose body about 1 μ diameter. It is possible that the latter is a developing conidium, but it is not quite what one would expect. Measurements of the conidia from nine heads gave the following: oval, ends acute, 3–5 × 1·5 μ (a small head); narrow oval or oblong-oval, ends obtuse, 5–8 × 2–2·5 μ; 4–7 × 2–3 μ (five synnemata from the same sporangium); 4–6·5 × 1·5–2 μ; 4–6 × 1·5–2 μ. It is possible that some of these were mixtures, as will be explained later. Chains of conidia were not observed.

Well-developed basal patches bore *Cephalosporium* conidiophores, up to 50 μ high, 2 μ diameter below, tapering regularly upwards. The *Cephalosporium* head, in general, was oval, about 10 × 7 μ, containing conidia in parallel, but some globose heads, with irregularly placed conidia, were found. The conidia in these heads were oblong-oval, obtuse, 5–8 × 2–3 μ. As a rule, these conidiophores arose from the white basal patch, quite independent of the regular glandular stalk. From a group of conidiophores round a broken *Stilbum* stalk, in which the conidia were 5–8 × 2·5–3 μ, one conidium was found, oblong-oval with obtuse ends, 12 × 4 μ.

In the following year (17. 10. 1931) two collections of *Stilbum tomentosum* on *Trichia varia* were made from the same log as the previous specimens. One was taken as possibly something different, as the *T ichia* sporangia were black and the *Stilbum* heads translucent when fresh, but the stalks were normally glandular and the conidia narrow oval, 3–5 × 1·5 μ, the larger with acute tips, the smaller obtuse. A culture was made from this gathering, and repeated transfers were made during the next five years. The *Stilbum* grew quite normally on oatmeal agar, with the typical glandular stalk and narrow oval conidia, with ends subacute, 3·5–5 × 1·5–2 μ, sometimes 6 × 2 μ.

In the second collection of the same date, the sporangia were yellow and the *Stilbum* heads opaque, many synnemata terminating in a small pencil of conidiophores with conidia, 3–4 × 1·5–2 μ. A *Cephalosporium* conidiophore from the basal mycelium of one synnema was 65 μ high, 1·5 μ diameter below, with conidia oblong-oval, 7–10 × 2–3·5 μ.

Seven other collections made at various times on *Trichia varia*, *T. affinis*, and *Perichaena populina* had conidia, 3–6 × 1·5–2 μ, occasionally 7 μ long. I have found the typical form with minute conidia on three occasions only, viz. on *Trichia decipiens*, Mulgrave Woods, 16 September 1930, conidia globose, 0·75–1 μ diameter, a few oval, 1 × 0·75 μ; on *T. persimilis*, Barnard Castle, 18 September 1933, conidia globose, about 1 μ diameter, or oval, 1·5 × 1 μ; on *T. affinis*, Duncombe Park, Helmsley, 31 August 1935, conidia globose, about 1 μ diameter, but some oval conidia, up to 9 × 3 μ also present. I regret that I did not take any of these into culture, probably because I was under the erroneous impression that I could obtain this form at any time nearer home.

In October 1934, gatherings of *Stilbum tomentosum* were made in Norfolk on *Cribraria rufa*, *C. argillacea* and *Comatricha pulchella*. In these, gathered in wet weather, the sporangia were bound together by mycelium. In one synnema, from *Cribraria argillacea*, the stalk terminated in a convex head,

surmounted by a distinct cap composed of moderately crowded *Cephalosporium* conidiophores, each with its separate globule of conidia, which were oblong, obtuse, sometimes curved, 5–7 × 1·5–2 μ. In another synnema from *Cribraria rufa*, *Cephalosporium* conidiophores were present on the stalk, with oblong or oblong-oval conidia, 4·5–7 × 2–3 μ. A culture was made on oatmeal agar from *Cephalosporium* conidia on the mycelium of the latter specimen. No *Stilbum* synnemata were produced in this culture, but scattered or crowded groups of *Cephalosporium* conidiophores, with a ring of separate conidiophores at the margin of the culture. The conidiophores were branched at the apex, with a single branch below, but not definitely acrostalagmoid; each branch bore a globule of oblong-oval, oval or cylindrical conidia, 5–11 × 2–3 μ.

Of nineteen recent British collections of *Stilbum tomentosum* made between 1930 and 1938, only three were the form which has been considered typical, that with minute conidia, either globose, 0·75–1 μ diameter, or oval 1–1·5 × 0·75–1 μ. The others were all var. *ovalisporum* A. L. Sm. (var. *macrospora* Ferr.), with oval conidia, usually 3–5 × 1·5–2 μ. Both forms are often parasitized by a *Cephalosporium*, which has oval, oblong-oval or cylindrical conidia, 5–12 × 2–4 μ, and the large spores which frequently occur in mounts of *Stilbum tomentosum* belong to the *Cephalosporium*. The stalks of both forms of *S. tomentosum* are minutely glandular as described by Greville; a tomentose stalk usually indicates that the specimen is parasitized. Extensive basal mycelium is usually that of the parasite, but unparasitized specimens have a slight basal mycelium, especially when old.

It would appear that Grimm cultivated the *Cephalosporium*, as his figures show *Cephalosporium* heads and he gave the dimensions of the conidia as 9 × 4 μ. His fungus was apparently a tuft of *Cephalosporium* conidiophores, not *Stilbum tomentosum*. Von Höhnel (1916) stated that *Tilachlidium* was a *Cephalosporium* coremium, but that is rather an over-simplification, unless he got his idea of the genus from Grimm's figure. In any case, Grimm's results do not warrant the transference of *Stilbum tomentosum* to *Tilachlidium*.

The descriptions of the genus *Tilachlidium* and its type species, *T. pinnatum*, are somewhat confusing, because Preuss described the *Cephalosporium* conidiophores as branches. Amalgamating the two descriptions, *Tilachlidium pinnatum* had a white or pallid simple clava, composed of agglutinated hyphae, covered everywhere with spreading *Cephalosporium* conidiophores, the conidia being ovate-oblong, hyaline, dimensions not stated; it occurred on effete agarics, especially *Mycena galericulata*. Miss A. L. Smith (1909) described another species, *Tilachlidium subulatum*, on plant debris, the clavae being gregarious, erect, yellowish or greyish white, covered everywhere with *Cephalosporium* conidiophores, which Miss Smith's figure shows more or less perpendicular to the clava, with cylindrical conidia, about 5–7 × 2 μ. From the descriptions these two species appear to be the same.

In 1937, I pointed out that *Isaria brachiata* (Batsch) Schum. was a *Tilachlidium*, but did not 'make the combination'. The name was published as *T. brachiatum* (Batsch) Petch in *Trans. Norfolk and Norwich Nat. Soc.* (1940), xv, 198. The fungus was originally described by Batsch (1786) as

Clavaria, and occurs principally on decaying agarics, but sometimes on vegetable debris, though there is a possibility that in the latter instances some unrecognizable agaric remains may be present. It has a wide distribution, and I have examined specimens from Britain (Yorks and Norfolk), the U.S.A. and Mauritius. The clavae usually occur in clusters, and are white to pale brown, simple or branched. As figured by Batsch, the branches sometimes leave the main stem at unusual angles and are narrowest at the point of attachment, while they are sometimes again similarly branched. The branches and the main stem are covered with spreading *Cephalosporium* conidiophores, simple or branched, usually perpendicular to the stem or branch. The conidia are cylindrical or narrow oval, hyaline, 2–7 (usually 3–6) × 0·75–1·5 μ. *Tilachlidium pinnatum* and *T. subulatum* appear to be merely the simple form of *T. brachiatum*.

Tilachlidium is not merely a *Cephalosporium* coremium, that is, it is not composed of hyphae which separate and curve outwards at different heights, each terminating in a *Cephalosporium* head. It has a solid stem, tapering to an acute apex, and composed of parallel agglutinated hyphae which do not separate. The *Cephalosporium* conidiophores are not hyphal tips, but arise perpendicularly from the exterior hyphae of the stem. Grimm's figure is not a *Tilachlidium*.

If the type specimen of *Corethropsis epimyces* Massee (1885) is available, it might be worth while examining it to ascertain whether it is not *Tilachlidium brachiatum*. It occurred on decaying *Mycena pura*.

Summary

Two forms of *Stilbum tomentosum* occur, one, taken as the type, with minute conidia, the other, var. *ovalisporum* A. L. Sm. (var. *macrospora* Ferr.), with conidia, 2·5–5 × 1·5–2 μ. The latter appears to be the commoner in England. Both have glandular stalks.

Both forms are frequently parasitized by a *Cephalosporium*, which has conidia, 5–12 × 2–4 μ.

Grimm cultivated the *Cephalosporium*. The fructification he obtained in culture was apparently a tuft of *Cephalosporium* conidiophores, not *Stilbum tomentosum* nor a *Tilachlidium*.

Isaria brachiata (Batsch) Schum. is a *Tilachlidium*, *T. brachiatum* (Batsch) Petch (1940). *T. pinnatum* Preuss and *T. subulatum* A. L. Sm. appear to be the simple form of *T. brachiatum*.

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INVESTIGATION INTO THE PRODUCTION OF BACTERIOSTATIC SUBSTANCES BY FUNGI

CULTURAL WORK ON BASIDIOMYCETES

By W. H. WILKINS, *Mycology Laboratory, University Department of Botany, Oxford*

Fungi which have produced bacteriostatic substances of therapeutic importance have usually belonged to the genera *Aspergillus* and *Penicillium*. In this Laboratory some 150 species and strains of the former and some 200 species and strains of the latter genus have been investigated with more or less gratifying results (Wilkins & Harris, 1942, 1943*a*, 1944*b*, 1944*d*). More recently, attention has been paid to the production of such substances by the larger Basidiomycetes and of the 780 species which have been examined about 10 % were strongly and about 20 % weakly antibacterial in action (Wilkins & Harris, 1944*c*). Though positive results were less frequent in the Basidiomycetes than in the two first mentioned genera there appears to be justification for further investigation. Species of *Aspergillus* and *Penicillium* present little cultural difficulty, they grow rapidly on a variety of solid and liquid media and can be tested with reasonable accuracy by the method devised by the author. The Basidiomycetes, on the other hand, present, in addition to the difficulty of isolation, special difficulties in culture. Many will not grow satisfactorily on any medium, solid or liquid, that has yet been tried; many are extremely specific to certain media; while many others grow so slowly as to be useless as a means of producing anti-bacterial substances in anything approaching a reasonable time. This paper deals with experimental work which attempts a solution of at least some of the difficulties involved. It has been assumed that the sporophores themselves could not be regarded as a reliable source of anti-bacterial substances in the therapeutic sense, hence the object was to induce the fungus to grow on a liquid medium and produce a metabolism solution which could be tested as to its effect on bacteria and from which the anti-bacterial substances (if any) could be chemically extracted, thus following the line adopted in the case of the 'moulds' mentioned above. Of the total number of 780 Basidiomycetes which had been tested as extract, fifty-six were isolated in culture. These were not specially chosen but just happened to be those which were available for collection and which were successfully grown in culture, and some had given a positive and some a negative result when tested as sporophore extract. These cultures were grown on a variety of different solid and liquid media but it was found that the media most favourable for growth were the three that had been found most satisfactory for species of *Aspergillus* (Wilkins & Harris, 1944*d*), viz. Potato dextrose, Malt and Czapek-Dox. Considerable attention was paid to methods for ascertaining rapidly whether a fungus might be regarded as promising

without, if possible, going through the lengthy process of growing it on the liquid medium. This will be referred to later. The methods which were used for testing the bacteriostatic properties of these Basidiomycetes were three. (1) Freshly gathered sporophores were squeezed or ground and the 'juice' or extract was tested by the 'hole' method mentioned above (Wilkins & Harris, 1943). This was the preliminary (and usually the only) test which was applied to the 780 species already tested. (2) The fungus was isolated in pure culture on an agar medium and tested by the mycelial 'disc' or 'strip' methods (Wilkins & Harris, 1944, 1944*a*). (3) The fungus, having been isolated, was grown on liquid media and the metabolism solution was tested by the standard 'hole' method already mentioned. Whether tested by the extract, strip or liquid methods, each fungus was tested against two representative types of bacteria, *Bacterium coli* and *Staphylococcus aureus*. In accordance with previous experience of the non-susceptible reaction of *Pseudomonas pyocyanea* to most Basidiomycetes, this bacterium was not used.

The following list shows the results of testing the fifty-six fungi which have been isolated as, (1) extract, (2) strip, and (3) liquid. In the last two cases, for the purposes of comparison, the fungi were grown on each of the three media mentioned. Sub-cultures of most of the fungi have been deposited with the Lister Institute and the number following the name of the fungus is the Lister Reference Number. In the list the symbol 'X' indicates a positive and the symbol 'O' a negative result. The symbol '—' indicates that mycelial growth was so slow that no test could be made within any reasonable time limit, and a complete blank means that no test was made. The letters PD, M and CD stand respectively for potato dextrose, malt and Czapek-Dox media. Under each test the results are in pairs, the first symbol in each pair represents the action against *B. coli* and the second symbol of the pair shows the action against *Staphylococcus aureus*, e.g. 'O.X' indicates negative against *B. coli* and positive against *S. aureus*.

List of fungi tested

		Strip			Liquid		
	Extr.	PD	M	CD	PD	M	CD
Armillaria mucida 6900	O.O	O.O	O.O	O.O	O.O	O.O	O.O
Boletus bovinus 6905	O.O	O.O	O.O	O.O	O.O	O.O	O.O
Clitocybe aurantiaca 6879	X.O	O.X	O.X	O.X	—.—	O.X	—.—
C. cerussata 6924	O.O	O.O	O.O	O.O	—.—	—.—	—.—
C. clavipes 6902	O.X	O.X	O.O	O.O	O.O	O.X	O.O
C. diatreta 6918	O.O	O.O	O.O	O.O	X.X	X.X	—.—
C. flaccida 6904	O.X	O.X	O.X	O.X	O.X	O.X	O.X
C. geotropa 6886	O.O	O.O	O.O	O.O	O.O	O.O	O.O
C. infundibuliformis 6913	O.O	O.X	X.X	O.O	X.X	X.X	—.—
C. inversa 6878	X.X	O.X	X.X	O.X	O.X	X.X	O.X
C. nebularis 6940	O.O	O.X	O.O	O.X	X.X	O.O	—.—
C. odora 6901	O.X	O.X	X.X	X.X	—.—	X.X	—.—
C. vibecina 6936	X.X	X.X	X.X	O.X	O.X	O.O	—.—
Clitopilus prunulus 6911	O.X	—.—	—.—	—.—	O.X	O.X	—.—
Collybia butyracea 6882	O.O	O.O	O.O	O.O	O.O	O.O	O.O
C. fusipes 6908	O.O	O.O	O.O	X.X	O.X	X.X	—.—
Coprinus atramentarius 6899	O.O	O.O	O.O	O.O	O.O	O.O	—.—

List of fungi tested (continued)

	Extr.	Strip			Liquid		
		PD	M	CD	PD	M	CD
C. comatus 6892	O.O	O.O	X.O	O.O	O.O	X.X	X.O
C. picaceus 6888	O.O	O.X	O.X	O.X	O.O	O.X	O.X
Crucibulum vulgare 6955		—.—	—.—	—.—	O.X	O.X	O.X
Flammula sapinea 6893	O.O	O.X	O.X	O.O	O.O	O.X	—.—
Hebeloma crustuliniforme 6938	X.X	—.—	—.—	—.—	O.O	O.O	O.O
Hygrophorus eburneus	X.X	—.—	—.—	—.—	X.O	O.O	—.—
H. pratensis 6954		O.X	O.X	X.X	O.X	O.X	O.O
Hypholoma fasciculare 6898	O.O	O.O	O.O	O.O	O.O	O.O	—.—
Lepiota procera 6894	O.O	O.O	O.O	O.O	—.—	—.—	—.—
L. rhacodes 6887	O.O	O.O	O.O	O.O	O.O	O.O	—.—
Marasmius alliaceus 6880	X.X	O.O	O.O	O.O	O.O	O.O	O.O
M. conigenus 6890	O.X	X.X	X.X	O.X	X.X	X.X	O.X
M. oreades 6906	O.O	O.O	O.O	O.O	O.O	O.O	O.O
M. peronatus 6885	O.O	O.O	O.O	O.X	O.O	X.O	—.—
Mycena galericulata 6937	O.O	O.O	O.O	—.—	—.—	—.—	—.—
Naucoria pediades 6909	O.O	X.O	O.O	X.O	O.O	O.O	X.O
Omphalia umbilicata 6920	X.X	O.X	O.X	O.O	O.X	O.X	—.—
Panus stipticus 6903	O.X	O.X	O.O	O.O	O.X	O.O	O.O
Paxillus involutus	O.O	—.—	—.—	—.—	—.—	—.—	—.—
Pholiota aegerita 6939	O.O	O.O	O.X	X.X	O.O	O.X	O.X
Pleurotus sapidus 6910	O.O	O.O	O.O	O.O	O.O	O.O	O.O
Polyporus betulinus 6891	O.X	X.O	O.O	O.O	O.X	O.O	O.O
Polystictus versicolor 6895	O.O	O.O	O.O	O.O	O.O	O.O	O.O
Psalliota arvensis 6889	X.X	X.X	X.X	O.O	X.X	X.O	X.O
P. campestris 6916	O.O	O.O	O.O	O.O	O.O	O.O	—.—
P. haemorrhoidaria 6915	O.O	—.—	—.—	—.—	O.O	O.O	—.—
P. pratensis 6925	O.O	O.O	O.O	O.O	O.O	O.O	—.—
P. sylvatica 6914	O.O	O.O	O.O	—.—	O.O	O.O	—.—
P. sylvicola 6912	O.O	O.O	O.O	—.—	O.O	O.O	O.O
P. villatica 6917	O.O	O.O	O.O	O.O	O.O	O.O	—.—
P. xanthoderma 6921	X.X	X.X	X.X	X.O	X.O	O.O	—.—
var. *grisea* 6923	X.X	X.X	X.X	X.O	O.O	X.O	—.—
var. *lepiotoides* 6897	X.X	X.X	X.X	O.O	X.X	O.O	O.O
var. *obscurata* 6922	X.X	X.X	X.X	O.O	O.O	O.O	—.—
Stropharia aeruginosa 6896	O.O	O.O	O.O	O.O	O.O	O.O	O.O
Tricholoma grammopodium 6877	O.O	O.O	O.O	O.O	O.O	O.O	O.O
T. nudum 6935	X.X	X.X	X.X	X.X	X.X	O.X	O.X
T. personatum 6876	X.X	X.X	X.X	—.—	O.X	O.O	—.—
T. sordidum 6919	O.X	X.X	X.X	X.X	O.X	O.O	—.—

From the above it is at once apparent that the action against *Staphylococcus aureus* is more frequent than that against *Bacterium coli*. It is in fact rare to find an action against *B. coli* only. This confirms previous findings.

It is also obvious that the choice of medium is an important factor. Potato dextrose and malt are, in general, equally suitable but rarely are both equally favourable for the same fungus. In most cases potato dextrose gives rather wider zones than does malt, though this fact is not indicated in the above results. Liquid Czapek-Dox gives poor results, many of the fungi will not grow on it and in any case the results on this medium are never as high as on either of the other two. Czapek-Dox agar, on the other hand, is more satisfactory all round, *Collybia fusipes* for instance, would not grow on the liquid but in the strip test the CD agar was the only medium that gave a positive result. Four years of experience have repeatedly suggested that the composition of the substrate, either as an artificial

medium or in natural habitat, is perhaps one of the most, if not the most, significant factor in the production of bacteriostatic substances by fungi, and this question is being systematically investigated.

The determination of a method to indicate whether a fungus is likely to produce anti-bacterial substances in quantity and in a reasonable time, though not wholly satisfactory, has met with some measure of success. It was at first hoped that a positive sporophore extract would give a positive metabolism solution and vice versa, but the following figures from the list show that this is not entirely reliable:

Numbers of fungi that are		
Positive in extract	Negative in liquid	Positive in liquid
22	2	20
Negative in extract		
32	22	10

Those that are positive in extract are, with two exceptions, which may again be due to medium complications, positive in liquid culture, but in the case of those which are negative in extract the chances of their being negative in liquid culture are only two to one, and it would be undesirable to lose possible good 'positives' to the extent of 30 % of those tested.

Because the above difficulty had been a constant source of anxiety the 'disc' and 'strip' methods of testing were devised. It was realized that there was considerable difference between what might be expected from a squeezed sporophore and what might result from the same fungus grown in culture, but it was hoped that between a fungus grown on an agar medium and the same fungus grown on a similar liquid medium, there would be some similarity of reaction. The following figures show some justification for this idea:

Numbers of fungi that are		
Positive in strip	Negative in liquid	Positive in liquid
28	0	28
Negative in strip		
28	24	4

Here it seems that if a fungus gives a positive result in the strip test it will give a positive result when grown in liquid and, if the strip test is negative, approximately 90 % of the liquid tests will be negative also. Though discouragingly large it might be that the discrepancy was due to experimental error but, in any case, this method, which has the advantage of being simple and rapid, is the most satisfactory yet devised.

The question of strain variation which was examined in *Aspergillus* (Wilkins & Harris, 1944*d*) presents itself here. It was found that individuals of the same species collected from different localities gave results which were constant for any given locality but which varied from strongly positive to negative according to the locality from which they were collected. As these results were based almost entirely on sporophore extract alone, further statement is postponed until investigations now in hand are completed.

Conclusions

The evidence to date suggests that the larger Basidiomycetes are significantly productive of the anti-bacterial substances and merit further investigation. It appears that, contrary to original expectation, the testing of the sporophore extract alone, though to some extent indicative, is not wholly reliable as a test for a positive result in the ultimate metabolism solution. It seems to be essential to isolate the fungus into culture when a strip test will, in the majority of cases, give a reasonably satisfactory indication as to what might be expected from liquid culture. Strain variation, i.e. in the present sense the variation of individual fungi, still requires elucidation and is being further investigated.

To my Research Assistant, Dr G. C. M. Harris, who has now left to take up another appointment, I am grateful for valuable help with the above work.

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A ROOT ROT OF CINERARIA, AND A STUDY OF THE SPECIES OF *PHYTOPHTHORA* CONCERNED

By MOIRA C. D. MUNRO, B.Sc. (Hort.), Ph.D.,
University of Bristol Research Station, Long Ashton

Introduction

Cinerarias were grown to a considerable extent in Ayrshire nurseries for the pot plant trade before the war. Many nurserymen annually lost an appreciable proportion of their stock as the result of a root-rot disease associated with wilting of the leaves. This disease appeared at all stages in the growth of the Cineraria, but the periods when plants were most susceptible were during the seedling stage and just prior to flowering.

Isolations made from fragments of diseased root tissue almost always resulted in a culture of a phycomycetous fungus resembling a species of *Phytophthora*.

Review of previous work

Few records exist of research into the cause of a Cineraria Root Rot. The only direct references to a naturally occurring Root Rot ascribed to *Phytophthora* are those of Pethybridge and Lafferty (1919) in Britain, and in Victoria (Australia) of Brittlebank and Fish (1927), who name as the causal organism *P. cryptogea*. Tomkins, Tucker and Gardner (1936), working on a Cauliflower Root Rot caused by *P. megasperma*, reported the same fungus present on Cinerarias affected with Root Rot. Symptoms of wilting were artificially induced in Cineraria by Tomkins and Tucker (1937) using an isolate of *P. cryptogea* from China Aster. In 1935 Drechsler recorded *Pythium* on Cineraria roots, though the fungus was not isolated.

Symptoms

The disease is first apparent when the aerial portions of the Cineraria show a tendency to wilt. The lower leaves are affected first, while still retaining their green colour; later the young leaves droop. First the laminae become flaccid, then the petioles loose their turgidity. The central axis of the plant usually remains upright. The condition appears suddenly in 1–2 days.

In severe attacks shading and watering failed to produce any recovery. When sun heat became more intense and pots dried out, some plants showed a tendency to wilt more readily than others. These were probably plants in which fungal invasion was still not far advanced, and they were capable of recovery when shade and water were provided. The infected plants, however, remained less tolerant of extremes than plants free from disease.

When wilted plants were shaken from their pots, a pinkish coloration of the roots, in contrast with the white roots of healthy specimens, was a

constant feature, though sometimes it appeared only in patches. In advanced stages of the disease the roots were brown and a soft odourless rot was evident. The cortex sloughed easily on pulling the root from the soil. In the crown of the plant, rotting of the xylem and pith was frequently involved.

These symptoms appeared on both *Cineraria stellata* and *Cineraria grandiflora.*

Isolation of the causal organism

Roots which showed the pinkish discoloration of the stele were selected from diseased plants at different stages of development. These were cut into short lengths, surface sterilized in 0·1 % mercuric chloride and plated on potato agar. Fungal growth was observed after 2–3 days.

The fungi isolated included species of *Fusarium* and on three occasions *Ascochyta*, but most of the platings produced, usually as the only growth, a phycomycetous fungus resembling either *Pythium* or *Phytophthora*. It is difficult to distinguish between these genera, but the presence in some of these Phycomycetes of amphigynous oogonia, and the tendency to release zoospores directly from the sporangium with no external vesicle, suggests that the genus is *Phytophthora*. From March and throughout the summer, all but one of the diseased plants whose roots were plated produced *Phytophthora* cultures.

In view of the ease and frequency of *Phytophthora* isolations, sometimes appearing as pure cultures, it seems most likely that isolates of this genus are the cause of Root Rot of the Cineraria.

Proof of pathogenicity

Artificial inoculations of healthy plants were carried out using pure cultures of several isolates, grown on crushed oats, cracked wheat, or potato agar. The inoculum was introduced into the soil near the roots of healthy plants. Injury to plant roots was avoided as much as possible by knocking the plants out of their pots and pushing pieces of culture into the side of the soil ball. As a rule 3–5 points of infection were introduced into the root ball of each plant in a $4\frac{1}{2}$ in. pot. The Cinerarias were then replaced in their pots without further root disturbance.

The plants, with controls, were kept in a slightly heated greenhouse and given ample root moisture.

As a result of these inoculations 64 % of the plants produced wilt symptoms and root rot. Some of the isolates attacked more readily than others but all were capable of producing symptoms of Root Rot. The disease usually became apparent in four weeks, and sometimes the symptoms could be distinguished in eight or nine days.

Roots from artificially infected plants were surface sterilized and plated on potato agar; these again yielded *Phytophthora* cultures.

Description of the isolated species

Five isolates of *Phytophthora* obtained from five wilted plants grown in Ayrshire were labelled F_2, G_1, H_1, J_2 and R_1.

These isolates when cultured on a variety of media, showed considerable variation.

On potato agar

On plates of this medium H_1 produced a prostrate spreading mycelium, hyaline and of an even density, and close, rapid growth. The hyphae were coarse, infrequently branching and occasionally septate; their diameter was 4–7 μ.

The mycelium of isolates J_2, R_1, F_2 and G_1 bore more resemblance one to another; all produced a small amount of thin aerial mycelium over the prostrate, spreading, hyaline growth. At 20° C. the increase in diameter was much slower than that of H_1. Microscopically the mycelia of J_2, R_1, F_2 and G_1 showed a close resemblance. Hyphae branched almost at right angles, and the young branches were of a similar diameter to the hyphae from which they arose. For the most part the hyphae were coenocytic, but septa were found in older hyphae of all four isolates. The average diameter of the hyphae was 5·5 μ, but this was not constant throughout the length. In some parts the hyphae appeared to be distended and in others constricted. A constriction occurred particularly at the point of origin of a lateral branch from its parent hypha. None of the cultures produced spores.

On oatmeal agar

Profuse growth developed on plates of oatmeal agar at 24° C. The aerial mycelium of isolates R_1, H_1, G_1 and J_2 was abundant, white and flocculent; that of F_2 was less plentiful. The incidence of reproductive organs was variable. The mycelia of F_2, R_1 and J_2 were purely vegetative after 6 week's growth. That of H_1 bore irregularly shaped granular bodies which might have been sporangia. The mycelium of J_2 bore thin-walled intercalary swellings.

In Petri solution

Tucker (1931) found that some species which rarely produce sporangia on solid media could be induced to form them in Petri solution. Tubes of this solution were inoculated with pieces of agar culture of F_2, G_1 and H_1. After incubation at 24° C. for five weeks the mycelia of F_2 and G_1 remained vegetative. In the culture of H_1 some very small bodies, 11 μ in diameter, resembling sporangia were found.

On plugs of Cineraria tissue

In the taxonomic keys relating to *Phytophthora* (Tucker, 1931; Leonian, 1934) the form of the antheridium, whether amphigynous or paragynous, is a major feature in classification. If sexual organs could be induced to form, they would prove a useful means of differentiation between the strains and an aid to identification of the species. Since the isolates showed no tendency to form reproductive organs on artificial media, it seemed possible that they required a substratum more closely resembling the host plant for their development.

Stems of *Cineraria stellata* were cut into convenient lengths to fit into test-tubes, and split lengthwise to expose the soft inner tissue on which the fungus may grow more readily. Sterilization was carried out at 1 atm. for 20 min. Two days later the plugs were inoculated with all five isolates and incubated at 24° C.

No growth was produced by J_2. Abundant flocculent mycelium formed in 4 days on plugs inoculated with H_1, in 6 days from R_1, in 10 days from F_2, and in 14 days a slight weft developed from G_1 with very small beaked or papillate sporangia.

Sexual organs were infrequent. The first to be observed were found on F_2, two weeks after inoculation of the plug. The oogonia were golden brown, smooth-walled and spherical, drawn out slightly within the amphigynous antheridia. The oogonia measured 25–29·7 μ in diameter or 37 μ to the base of the antheridium. Oospores measured 18·6–22·3 μ diameter. A later inoculation failed to produce oogonia until after a 13-week interval when the plug had almost dried out. Oogonia were then found in abundance.

The only other isolate to form oogonia was R_1. On this culture after 5 weeks there were many golden brown oogonia, 25·5–30 μ diameter, with amphigynous antheridia. The other isolates showed no sexual stage.

Sporangial formation

The transference of the fungus from a highly nutritive substratum to a liquid medium of low nutritive value has been found to have the effect of inducing members of this genus to form sporangia (Pethybridge & Lafferty, 1919). This method was applied to the isolates from Cineraria in three ways.

(1) Well-nourished aerial hyphae were taken from agar cultures, avoiding where possible any fragment of the agar substratum. F_2, G_1 and H_1 were taken from oatmeal-agar cultures and J_2 from potato agar. Tubes of sterile distilled water were inoculated with this vigorously growing material.

In 2 weeks' time H_1 had formed spherical thin-walled sporangia in abundance. F_2 produced sporangia after seven weeks more typical of the genus *Phytophthora*. These were elongate-ovoid, somewhat irregular in outline, measured 22·2–15 × 11–7·5 μ, and had granular contents. The other isolates, G_1 and J_2, remained vegetative. Oval intercalary chlamydospores were found on the mycelia.

(2) Five isolates were grown on a highly nitrogenous medium consisting of potato agar to which 1 % KNO_3 had been added (pH 5·5). After a few weeks' growth, subcultures were made in liquid bog soil extract (pH 4·6). The tubes were incubated at 20° C. for three weeks before examination.

Isolate F_2 under this treatment produced no sporangia comparable with those induced by transference to distilled water. A few intercalary swellings were present. G_1 bore a few small ovoid sporangia on very strong hyphae. Intercalary swellings of this isolate were seen to be germinating in situ, one or more hyphal tips emerging from all sides of a single swelling. J_2 formed large ovoid sporangia, quite distinct from the intercalary swellings which were also present and which germinated similarly to those of G_1.

R_1 and J_2 both formed large ovoid sporangia in culture; those of R_1 measured $44{\cdot}4 \times 25{\cdot}8\mu$. Intercalary swellings germinating in all directions by hyphal protuberances were present in groups. H_1 produced no true sporangia, but some short lateral hyphae were abnormally swollen, making 'thumb-like' projections from the main hyphae.

(3) Leonian's technique (1934), involving the transference of cultures from pea broth to distilled water, was followed in order to encourage sporangial development in the present isolates, with these results:

F_2. Occasional sporangia, oval non-papillate; av. $30{\cdot}6 \times 21{\cdot}6\mu$.

J_2. Occasional sporangia, oval non-papillate; av. $31{\cdot}5 \times 21{\cdot}5\mu$.

H_1. Round intercalary sporangia.

R_1. Vegetative.

From the cultural work it was apparent that the isolates varied widely in their reaction to different media. Although a *Phytophthora* species could be isolated without difficulty from Cineraria plants with wilt symptoms, in each case an apparently different strain was obtained. Each showed distinctive reactions to cultural treatments distinguishing it from other isolates.

Similarly, when reisolates from artificial inoculation were grown in culture, it was found that some of these differed slightly in behaviour one from another and also from the strains which were introduced into the plant, yet all were apparently species of *Phytophthora.*

The presence of ovoid non-papillate sporangia of the type associated with the genus *Phytophthora* has been recorded for F_2, G_1 (very small), H_1 (very small), J_2 and R_1, though each produced the organs under different conditions. Oogonia with antheridia were found only in isolates F_2 and R_1.

The marked dissimilarity in behaviour suggested that more than one species may be present on Cinerarias affected with Root Rot. Reproductive structures, however, were so few and infrequent that it was difficult to identify the species by morphological features alone.

So far the isolates seem to fall into three groups:

F_2, R_1. Each formed oogonia with amphigynous antheridia and had large sporangia.

J_2. Similar sporangia were present but no growth developed on Cineraria plugs. The sexual stage was not observed.

G_1, H_1. These isolates produced very small sporangia.

Attempts to classify the Cineraria isolates

Keys to identification compiled by Tucker (1931) and Leonian (1934) are based on (*a*) the response of the organism to culture on certain media, notably by the production or absence of sexual organs or sporangia; (*b*) the tolerance of the species to extremes of temperature or concentrations of malachite green; and (*c*) differential pathogenicity on potato tubers.

Cultures on various media have been made and the resulting growth compared in a previous section. Subsequently experiments were carried

out to determine the pathogenicity on potato tubers, and the tolerance of the isolates of varying temperature and of concentrations of malachite green. In addition, trials were made to indicate the range of *p*H which would support the growth of the various isolates.

Pathogenicity on potato tubers of *Phytophthora* isolates from Cineraria

Cultures of the fungus on potato agar were used for inoculation of the tubers. Potatoes were washed and, after surface sterilization with alcohol, three V-shaped incisions were made equidistantly spaced on three sides of each. The flap of tissue so made was raised and a piece of agar inoculum bearing the *Phytophthora* was introduced underneath. Each potato was thus inoculated three times with the same isolate. Controls were tubers similarly cut but not inoculated. The tubers were kept moist under inverted sterilized bell jars and incubated at 24° C.

Results

J_2. After 6 days the tissue surrounding all inoculated lesions had turned brown and rotted, while all control cuts remained healthy. The isolate was pathogenic to potato tubers and was reisolated from the lesions.

R_1. Two inoculations proved pathogenic in seven days. All controls remained healthy.

F_2. All inoculations proved pathogenic after seven days.

G_1, H_1. After 20 days, inoculated cuts still remained healthy. A thin callus formed on the tissues lining the cut. These two isolates were inoculated into a second series of tubers and again they were non-pathogenic.

The relationship between temperature and growth of Cineraria isolates

Plates of potato agar (*p*H 5·8) were inoculated with the five strains of *Phytophthora* derived from Cineraria plants. The inoculum was of uniform size and planted in the centre of the plate to allow of equal growth on all sides. The plates were incubated at temperatures ranging from 16 to 35° C. Once set, the incubators maintained a fairly steady temperature.

Recordings of the mean diameter of growth were made after 96 hr. For each strain there is an upper temperature limiting to growth. F_2 was inhibited at 30° C. G_1 and J_2 produced a trace of growth at 30° C. but failed to grow at 32° C. R_1 gave measurable growth at 30° C. but also failed to grow at 32° C. H_1 made vigorous growth at 32° C., but at 35° C. was inhibited. In each case the limiting effect of increased temperature acted rather suddenly; vigorous growth was brought abruptly to an end by an increase of 1–2°.

The effect of low temperatures was also tried. In this case potato-agar plates were incubated at 24° C. for one day, in order that initial growth might develop in the fresh agar. The circumference of growth was outlined on

the under glass of the Petri plates before the series was transferred to a cold room, with a temperature of 8–9° C.

The growth of H_1 at this low temperature was the most rapid. F_2 grew slightly. The development of R_1 and J_2 was scarcely perceptible.

Differential growth in malachite green solution

Leonian (1934) found that the response of different species to the presence of small amounts of malachite green in the growth medium was a fairly constant specific character on which classification within the genus might be based. His cultures were in a liquid medium, the formula for which was closely followed in this work except for the substitution of Witte peptone for the proteose peptone used by Leonian. His technique for dissolving in it minute quantities of malachite green was also employed. Dilutions containing 1 part of dye in 2, 3, 4, 8 and 12 millions were prepared. Tubes were selected for uniformity in diameter and shape. The media were tubed (5 c.c. per tube) and sterilized, and after a few days were inoculated. Inoculum was cut from potato agar plates of the various Cineraria isolates, using a sterilized platinum wire bent to form three sides of a 1 mm. square. In this way the size of inoculum used in each tube could be made nearly identical. One piece of inoculum was introduced into each tube, and each treatment repeated four times. The tubes were kept at 20° C., and after two weeks they were examined and the amount of growth measured and recorded.

The concentration of 1 in 3 millions prevented the growth of all the isolates. F_2 and G_1 would tolerate a concentration of 1 in 4 million. J_2 was more sensitive, and only one plate at the concentration of 1 in 8 million showed a trace of growth; even at 1 in 12 million growth was slight. H_1 was the most sensitive of all, even the most dilute solution of the dye inhibited its growth.

Review of results

A study of the foregoing experiments dealing with the effects of temperature and different concentrations of malachite green on growth, and with pathogenicity for potato tissue, indicates that there is a tendency, among the isolates of *Phytophthora*, to group themselves in a manner which is repeated in each experiment. Isolates J_2, F_2 and G_1 repeatedly show a very similar growth response to a range of temperatures. H_1 produces wider growth and tolerates higher temperatures than other isolates.

On malachite green media, again the responses of isolates F_2, G_1 and J_2 approximate more closely than the others. R_1 resembles this group, but in a later experiment R_1 was slightly more tolerant than other isolates in that group. H_1 is distinct from all the others.

In pathogenicity tests on potato tubers, once more the resemblance held between F_2 and J_2. Isolate R_1 joins this group, and these produce disease within 7 days. H_1 was non-pathogenic. G_1, usually with the former group, this time falls into the latter, as it has been found to be non-pathogenic.

A further series of experiments dealing with the response of the isolates

to varying degrees of acidity was carried out to find if a similar grouping exists under these conditions.

The effect of varying pH on the Phytophthora *isolates from Cineraria*

Potato-agar medium of a series of *p*H values was prepared, adjusting the acidities by means of solutions of $Na_2HPO_4.12H_2O$ and pure citric acid to obtain the range of *p*H values 3·67, 4·19, 4·59, 5·96, 6·39, 6·68 and 7·17. Three plates of each *p*H value were inoculated with each of the *Phytophthora* strains F_2, J_2, H_1 and R_1 and incubated for four days at 23° C. At the end of that time the diameter of growth was measured and the results tabulated.

From a study of the results it appeared that over a wide range of *p*H, the effect on strains F_2, R_1 and J_2 was small. With a 2·3 increase in acidity (*p*H 6·4–4·1) the decrease in diameter growth was no more than 4–5 mm. As the acidity increased beyond *p*H 4·1 there was a sharp decrease in growth. At *p*H 6·68 there was a decrease in the growth of all isolates, but it increased again as the *p*H exceeded 7·0. The growth of H_1 filled the plate at *p*H values from 4·5 to neutrality.

Again the grouping of the isolates follows the previous tendency, F_2, J_2 and R_1 showing similarity in behaviour and H_1 behaving independently. In a previous experiment in which G_1 was included the graph for the development of G_1 closely followed that of isolates F_2, J_2 and R_1.

Reinoculation of Cineraria plants

The cultural work carried out with isolates of *Phytophthora* from Cineraria suggests that these include more than one species. The behaviour of isolates F_2, J_2 and R_1 show so close a similarity under all treatments given as to be considered a single species. G_1 reacted in the same way to temperature, acidity and the presence of malachite green, but was non-pathogenic on potato tubers. Except for this one response the strain resembled the previous ones. H_1 differed consistently from other isolates.

In order to determine whether one or all of these groups is responsible for the disease, artificial inoculations of healthy plants were made at intervals during all seasons of the year.

The results of the inoculations are here tabulated.

	Successful inoculations		Delayed symptoms				
Strain		Incub. days		Incub. weeks	Unsuccessful inoculations	Total	% success
R_1	10	7–32	1	15	—	11	100
J_2	5	6–25	3	6–17	1	9	89·0
F_2	8	8–28	—	—	4	12	66·7
G_1	5	8–40	2	6–14	4	11	63·6
H_1	3	17–35	1	10	7	11	36·3

R_1 proved to be the most virulent strain. J_2, F_2 and G_1 produced symptoms less readily, and H_1 occasionally produced disease but was less virulent.

Identification of the strains

An attempt was made to determine the species of the five isolates of *Phytophthora* by means of the keys drawn up by Tucker (1931) and Leonian (1934). To these were related the results of cultural work already described in this paper.

R_1. The isolate R_1, of which the life history is most complete, showed a spreading growth on agar media at 20° C., and the maximum temperature for growth was 30–31° C. No oogonia developed on oatmeal agar or on any other agar medium tested, but they appeared, with amphigynous antheridia, on tissue plugs. Sporangia, formed in a soil extract, were non-papillate.

From these features the fungus might be identified, according to Tucker (1931), as one of the following species:

P. erythroseptica, *P. Richardiae*,
P. Cinnamomi, *P. cambivora*,
P. cryptogea.

It can be distinguished from *P. erythroseptica* by its smaller oospores, i.e. less than 30μ in diameter. Its pathogenicity on potato tubers, its smaller sporangia, and optimum growth below 25° C., distinguishes it from *P. cambivora*.

The pathogenicity on potato tubers and absence of oogonia on oatmeal agar distinguish it from *P. Richardiae*. There remains, therefore, *P. cryptogea* and *P. Cinnamomi*.

Intercalary swellings developed in a culture transferred to bog-soil extract. The presence of these vesicles, rare in *P. cryptogea*, together with the lack of sexual organs on oatmeal agar, suggest that the isolate is *P. cinnamomi*.

Transference of a pea-broth culture to distilled water failed to produce any sporangia or sexual organs, though some hyphal swellings developed. Restricted growth continued in the presence of 1 in 8 million malachite green. From these additional features Leonian (1934) would classify the isolate as *P. Cinnamomi*. Thus the previous conclusion, based on Tucker's classification, is confirmed.

J_2. The behaviour of J_2 closely resembled that of R_1. Temperature relations were the same. Aerial mycelium formed on oatmeal agar and no oogonia were observed. Sporangia were of similar dimensions to those of R_1. Intercalary vesicles, germinating by hyphal tips, formed in bog-soil extract cultures of J_2 also. J_2 was pathogenic on potato tubers. These two isolates might be regarded as the same species, but oospores of J_2 were not observed. Provisionally, therefore, J_2 will be classed with R_1 as *P. cryptogea* or *P. cinnamomi*.

The nature of growth of distilled water after three days in pea broth, on which Leonian based his key, does, however, reveal a difference between J_2 and R_1. J_2 produced a very few scattered sporangia. This feature points to the isolate being *P. citrophthora* (Leonian, 1934). This is unlikely since the sporangia of the isolate are non-papillate, and the temperature relations recorded by Tucker (1931) do not agree. On account of the rare occurrence

of these sporangia J_2 might well be classed with the group in which they are absent, thus resembling R_1. It would then be classified as *P. cinnamomi.*

F_2. The temperature relations and the behaviour of this isolate on potato agar were similar to those of R_1, and it was pathogenic on potato tubers. Non-papillate sporangia and oogonia with amphigynous antheridia were formed. The oogonia measured 25–30 μ in diameter. Hence, according to Tucker (1931), the identity of the isolate might be:

P. cryptogea, *P. Richardiae*,
P. cambivora, or *P. Cinnamomi.*

From *P. Richardiae*, the isolate can be distinguished by temperature relations (*P. Richardiae* failed to grow at 10° C. (Tucker, 1931)), by pathogenicity on potato tubers, and by the size of oogonia (those of *P. Richardiae* are greater than 35 μ in average diameter).

The pathogenicity of F_2 on potato tubers, together with the smaller sporangia and its optimum growth below 25° C., distinguish it from *P. cambivora.*

The dimensions of sporangia and oogonia found in F_2 cultures conform more closely to *P. cryptogea.* The absence of oogonia on oatmeal-agar cultures, however, and the presence of groups of vesicles on cultures of F_2 suggest that this isolate again is *P. Cinnamomi.*

According to Leonian's key, the production of sporangia in sterile water after transference from pea broth together with the toleration of 1 in 8 million malachite green would classify this isolate as *P. citrophthora.* Again, as with isolate J_2, it is probable that since the production of sporangia is occasional, the fungus might be identified as *P. Cinnamomi.*

G_1. The behaviour of the vegetative mycelium of G_1 was very similar to that of R_1 and J_2. No aerial growth was formed. The temperature range was the same as that of R_1 and J_2. Intercalary vesicles, germinating by hyphal tips, were again present. In the reproductive structures no similarity could be traced. No oospores were observed, and the only sporangia which could be induced were very small. The isolate was not pathogenic on potato tubers. It is suggested that the rarity of sporangia and oogonia, the occurrence of vesicles on a few occasions only, and the non-pathogenicity of the isolate on potato tubers, place it near to *P. cambivora.* Its optimum temperature for growth is, however, much lower than that of *P. cambivora.* The identity of G_1 is therefore doubtful. Apart from pathogenicity on potato tubers, G_1 most closely resembles the isolates described above and identified as *P. Cinnamomi.*

H_1. The temperature range of isolate H_1 alone distinguishes it from all the other Cineraria isolates. It produced abundant and rapid growth from 20 to 32° C., but no growth at 35° C. Oogonia, sporangia and chlamydospores were not observed. The isolate was non-pathogenic on potato tubers.

Relating these features to Tucker's key (1931), the identity of this isolate might be *P. cambivora*, which is described briefly by Tucker as follows: 'Hyphae on agar media usually sterile. Vesicles occasionally developing, and oogonia rarely. Non-pathogenic on potato tubers. Optimum growth temperature is between 25 and 30° C. No growth occurred at 35° C.'

This description supports the probable identity of the isolate with *P. cambivora*.

H_1 formed no sexual bodies on transference from pea broth to sterile water. It was markedly intolerant of the most dilute solution of malachite green. Hence Leonian (1934) would group it as *P. cambivora*, *P. Colocasiae*, or *P. Porri*.

P. Porri ceases growth at a temperature below 31° C. H_1 continued growth when the temperature was reduced to 8° C. *P. Colocasiae* does not grow at so low a temperature. The papillate and pedicellate sporangia characteristic of *P. Colocasiae* were not observed. The identity of the isolate as *P. cambivora* is therefore confirmed. Thus there appears to be at least two well-defined species involved in the Root Rot of Cineraria:

P. Cinnamomi: isolates R_1, J_2, F_2.
P. cambivora: isolates H_1.

In addition, G_1 has not satisfactorily been classified under existing schemes.

The causal organism

Inoculation experiments have shown that symptoms of Root Rot of Cineraria were induced, within a few weeks, by the introduction into the surrounding soil of isolates R_1, J_2, F_2 and G_1. R_1, J_2 and F_2 have since all been identified as *P. Cinnamomi*. G_1 very closely resembles this species except for pathogenicity on potato tubers. The percentage successful infection induced by isolate H_1 was much lower. H_1 was identified as *P. cambivora*.

From the present study it would appear therefore that *P. Cinnamomi* is responsible in greatest measure for Root Rot in Cineraria. *P. cambivora*, though parasitic, is less virulent than *P. Cinnamomi*.

Summary

1. The symptoms of a Root Rot of Cineraria are described and a number of fungal isolations from diseased plants have been studied.

2. Considerable differences and variations were observed between these isolates, yet all apparently belonged to the genus *Phytophthora*.

3. Five isolates, from a variety of sources, were selected for more detailed experiments.

4. The isolates were cultured on several solid and liquid media, and their growth characters compared.

5. The influence of temperature, *p*H, and a range of concentrations of malachite green on the isolates has been studied.

6. Each Cineraria isolate was identified by its growth and response to various treatments, and finally, in spite of apparent considerable variation, most of the isolates were identified as *P. Cinnamomi*.

7. Inoculations of Cineraria plants showed that the three isolates identified as *P. Cinnamomi* were those which most rapidly produced symptoms of Root Rot. *P. cambivora* was a less virulent parasite.

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NEW AND INTERESTING PLANT DISEASES

By W. C. MOORE, *Plant Pathology Laboratory, Harpenden*

(With Plates VI and VII)

16.* Scale spotting of tulip bulbs

On 23 June 1941 Mr E. R. Wallace sent me some diseased specimens of tulip bulbs, variety Yellow Prince, that had been dug the day before from a fine, sandy soil near Spalding. The bulbs were of 12–14 cm. size and the outermost scale of each, though turning brown, was still somewhat fleshy and immature. Removal of this outer scale revealed brown markings on the first fleshy scale below. Many of the markings were pale, brown, indefinite, isolated spots a few millimetres across, which had coalesced where they were numerous to give irregular patterns on the surface of the scale. On some bulbs there were large, slightly sunken, rounded or heart-shaped areas with definite margins on the 'cheek' of the bulbs, and sometimes the whole of the tissues around the neck had a pale scalded appearance and stood out in marked contrast to the white portions of the scales. In a more advanced stage these areas had become patterned in light and dark brown rings and streaks (Pl. VI, fig. 1). The brown markings penetrated into the scale, but rarely, if ever, extended right through to its inner surface: on the other hand, similar but fewer markings were occasionally evident on the outer surface of the next inner scale. The trouble seemed to be restricted to the bulbs of plants that were still green or partially green, and was not noticed where the foliage had already died down completely.

The precise cause of this scale spotting has not yet been determined. Exactly similar symptoms were seen on tulip bulbs, also of the variety Yellow Prince, received from Lincolnshire in early July 1934. On that occasion the greater part of a stock lifted from a silt loam was affected. The bulbs had flowered well, but after lifting and before cleaning they were left in the open for a week in trays stacked ten to twelve in a pile. The period was one of hot sunshine and the top trays were unprotected, but these showed no more and no less of the trouble than the rest, and other adjacent varieties (Ibis and Brilliant Star) exposed in the same way were unaffected. Efforts to induce the condition in healthy bulbs by exposing them for a week to hot sun were unsuccessful. No fungus could be found in the spots, or be isolated from them, and although a bacterial organism, culturally very similar to *Bacillus coli* Migula, was isolated, infection experiments with it gave inconclusive results, and attempts to inoculate healthy bulbs with small pieces taken from affected ones were unsuccessful. One or two of the bulbs were sent to Dr E. van Slogteren, of the Laboratorium voor Bloembollenonderzoek at Lisse, who said he was familiar with the

* Previous contributions to this series were published in the *Transactions*, vols. xxii–xxvi.

trouble and regarded it as physiological in origin. He had already seen it that year in Holland on Yellow Prince and certain other varieties, and in his experience it was usually most severe in the best stocks and heaviest bulbs.

Attempts to isolate an organism from the bulbs received in 1941 also failed and the disease was not seen again until August 1944 when it was found on the variety Miss Blanche in Lincolnshire. It was then reported that the trouble occurs in that county to a greater or less extent each year in a number of varieties, is sometimes a source of very heavy loss, and is apt to be worse when the bulbs are lifted under wet conditions and are stored too thickly in warm, badly ventilated sheds.

17. Ring rot of green walnut fruits

On 20 September 1941 some green walnut fruits were brought to me which showed injury quite distinct from that caused by rooks. The fruits had fallen prematurely from two old, large trees growing in a private garden in Harpenden, and much of the fruit remaining on the tree was also clearly affected. Some fruits showed merely a small black, sunken area at the stigmatic end of the green husk, while in others the chief symptom was a relatively inconspicuous and roughly circular patch about an inch in diameter on one side of the husk immediately below the old stigmatic remains (Pl. VI, fig. 2). The tissues comprising this patch were uniformly raised above the level of the rest of the husk or were irregularly raised in a series of humps or corrugations: they were paler than the remainder of the husk and frequently showed a yellowish tinge. The boundary between the healthy and affected parts was marked by a wavy and often interrupted black band about two millimetres wide. Later, this blackened band broadened unequally and became a more or less complete ring; it also became sunken and showed a number of transverse cracks. Although the green portion within the ring projected well above the general level of the fruit at first, later it gradually became black and sunken and ultimately the green husk showed a uniformly black or blackish sunken patch, an inch or more across, on one side of the fruit. As the tissues blackened they became sprinkled over with orange-coloured sporodochia of a species of *Fusarium*. On a few fruits there was a patch on each side of the husk and occasionally the whole of the stigmatic end was involved.

The affected fruits were mostly immature and the shell within still relatively soft. On cutting through fruits still in the typical 'ring' stage, the tissues of the green husk around the remains of the stigma were found to be completely disorganized, as also were those in a narrow belt immediately below the black ring, whereas there appeared to be nothing abnormal about the tissues immediately below the green portion within the ring. The upper part of the shell below the remains of the stigma was yellow-brown and decayed for a distance of one centimetre or more from the tip, and was covered with a fine weft of cottony mycelium. The kernel itself appeared to be unaffected. In the later stages of attack the rot spread down the shell until the whole of it was brown and rotten. Before this

happens the central placental tissue becomes rotten. The kernel may remain healthy until the whole shell is decayed but sooner or later the skin covering it blackens and the flesh is reduced to a soft, yellow-brown mass.

Preliminary examination of the *Fusarium* occurring on the surface of the blackened fruits showed that the spores were predominantly 3–4-septate, with a range of dimensions of 23–33 × 3–4 μ for the 3-septate, and 30–39 × 4–5 μ for the 4-septate spores. Pure cultures were obtained from the sporodochia and the same fungus was isolated, though not consistently, from the decayed portion of the shell, but it was not possible to continue the observations at the time and the disease has not been encountered again. The specific identity of the *Fusarium* and its causal relation to the disease is therefore uncertain. Wollenweber (1931) has reported *F. lateritium* Fr. on walnut fruits in Germany and he considered it might well be parasitic on this host, as it is on a number of other fruit trees. He also mentioned that species of *Fusarium* had been reported on walnut fruits in France and Italy. Later, Wollenweber and Reinking (1935) listed three species—*F. lateritium* Fr., *F. sambucinum* Fuckel, and *F. avenaceum* (Fr.) Sacc.—as the cause of an uncommon fruit rot of walnuts. According to these authors *Fusarium* produces black spots at the flower end of the still green husks and penetrates, along with other fungi, into barren nuts: if the fruit stalks are implicated the fruits fall prematurely and keep badly.

18. Leaf spot of spinach (*Heterosporium variabile* Cooke)

In all probability this disease is much more widespread in Britain than the three existing records of it suggest. *Heterosporium variabile* was first recorded and named by Cooke (1877), who observed it on spinach leaves collected near Welshpool, and it was not reported again until Glasscock and Ware (1939) found it in May 1939 causing severe damage to 2½ acres of Prickly Seeded spinach sown the previous August in two plots in a market garden at Appledore, Kent. One plot sown in October and three others in the spring of 1939 were unaffected.

In May 1944 I received spinach leaves attacked by this fungus from Wimborne, Dorset. They represented two stages of a disease that had appeared about mid-April on a crop of the variety Nobel derived from imported seed sown early in September 1943. At first the appearance of the small white spots had suggested hail damage but on 23–24 April the trouble spread very rapidly over an acre of the crop. Spring-sown spinach of the same variety less than 100 yards away remained unaffected, and by mid-June three spring-sown crops of Nobel, in part from the same seed sample as that sown the previous autumn, had been cleared without showing signs of Leaf Spot.

Leaves in the early stages of attack showed small, sharply defined, rounded, white spots, 1–3 mm. across, scattered, but mostly on the upper half of the leaf, surrounded by a very narrow shrivelled or water-soaked band, and free from conidiophores and conidia of the *Heterosporium* (Pl. VII, fig. 3). In a more advanced attack there were many spots which were often coalescent, crowded towards the tip of the leaf, and covered or partly

covered with dark, olive-green or black spore tufts (Pl. VII, fig. 4). The leaves were turning brown and withering from the tips, and in the final stages some of them were almost covered with small, separate, dark fungus mats 1–3 mm. across. The fungus was at first mainly epiphyllous, then more or less equally abundant on both sides of the leaf.

19. *Botrytis* sclerotia in sainfoin seed

Mr C. C. Brett has informed me that on a number of occasions during recent years one or two of the seeds in certain sainfoin seed samples examined at the Official Seed Testing Station at Cambridge have developed black sclerotia during germination tests. Three such samples were met with in the early part of 1944 and Mr Brett kindly sent me several of the affected seeds. They were dead, with the contents mostly reduced to an amorphous mass. Little or nothing was left of the external parts except a skeleton network of the more resistant tissues, in which a number of black sclerotia were embedded. These sclerotia were individually about 3 mm. long, sausage-shaped, curved or irregular, depending partly on their position in the substratum. Each consisted of a very narrow, black rind, sometimes only a cell or two thick, surrounding a mass of interlacing hyphae. Plate cultures prepared from the sclerotia gave a scanty, but uniform, white to grey, aerial mycelium and a few erect conidiophores bearing whorls of conidia. Sclerotia varying in size and shape from rounded bodies 2–3 mm. across to elongated masses up to 1½ cm. long and ½ cm. wide, were produced in a ring near the edges of the plates: they were white at first but soon became black. The fungus was identified as a strain of *Botrytis cinerea* Fr.

20. *Phytophthora infestans* (Mont.) de Bary on *Lycium halimifolium* Miller

The following note is adapted from a memorandum kindly placed at my disposal by Dr G. H. Pethybridge, who prepared it in 1942 from observations made by him at the time.

Towards the end of the very wet August of that year Dr Pethybridge noticed that many of the leaves on the pendulous branches of a box thorn (*Lycium halimifolium* Miller) growing in a private garden at Bodmin (Cornwall) were brown and withered at the tips. Many also had one or more fairly large, roundish, brown spots on the lamina further back, sometimes reaching the margin and sometimes not. These leaves were being shed prematurely and readily fell off if touched. A large number of the abundant crop of bright red berries, and the calyx to which each was attached, were also affected. It was suspected that *Phytophthora infestans* (Mont.) de Bary might be the cause of the lesions, especially as potato Blight had been extremely prevalent in the neighbourhood. No fungus was visible on or around the lesions on the leaves still on the shrub, but when affected leaves were placed for twenty-four hours or so in a damp dish a copious halo of *P. infestans* grew out from the still green tissue surrounding the brown lesions, just as occurs with potato foliage spotted with

Blight. Moreover, this fungus was found surrounding the lesions on some of the damp fallen leaves and it was present also on some of the fruits.

No healthy, unsprayed potato foliage was available for cross inoculation purposes, but slices of healthy tubers were inoculated with spores from the *Lycium* leaves and berries, and suitably incubated at room temperatures. Within a day or two copious growth of *Phytophthora infestans* occurred on them, while uninoculated slices under the same conditions remained unaffected. An attempt to inoculate healthy *Lycium* leaves and berries with spores from blighted potato leaves gave inconclusive results: the leaves developed lesions similar to those on the growing shrub, but they soon became covered with mould growths and sporulating *Phytophthora infestans* could not be detected with certainty. The inoculated fruits did yield the fungus, but so did two out of three of the uninoculated controls. There was no reasonable doubt, however, that the attack on the shrub was due to spores from blighted potato foliage, which was abundant in the particular garden as well as in adjoining ones. The shrub concerned was growing in a position where temperature and atmospheric conditions must have been ideal for the attack.

The shrub affected was identified by Mr A. D. Cotton as *Lycium halimifolium* Miller (syn. *L. barbarum* Aiton non Linn., *L. vulgare* Dun., and *L. europaeum* Gouan non Linn.), which is said to be very closely allied to and scarcely specifically distinct from *L. chinense* Miller. *Phytophthora infestans* has been recorded from time to time on various Solanaceous hosts but only once before on *Lycium*. Vowinckel (1926) found it occurring naturally on *L. halimifolium* and succeeded in artificially infecting this species and *L. turcomanicum*. Although the present case, therefore, does not constitute a new host record for *Phytophthora infestans* it appears to be the first instance in which the potato Blight fungus has been reported on a species of *Lycium* in the British Isles.*

21. A SEEDLING DISEASE OF CELERY CAUSED BY *ALTERNARIA RADICINA* MEIER, DRECHSL. & EDDY

In April 1945 some diseased celery seedlings an inch or two high were received at the Plant Pathology Laboratory from a nursery at Eastrea in Cambridgeshire. They formed part of a large batch of seedlings raised from seed that had probably been derived from plants raised from imported seed. Another large batch of seedlings grown in the same soil but derived from a different source of seed was not affected. The diseased seedlings were examined by Dr Alex Smith, to whom I am indebted for permission to summarize his observations. He found that, although the seedlings presented all the symptoms of Root Rot caused by *Phoma apiicola* Kleb., there was no sign of the pycnidia of this fungus on them or on a sample of the seed from which the seedlings had been raised. He therefore made isolations from the material, and some of the affected seedlings, as well as several hundreds of the seeds, were placed on moist filter paper in Petri dishes and incubated for a few days at room temperatures. Pure cultures

* The record listed by Moore (1943, p. 12) is the one dealt with here.

were obtained of a fungus which could not be distinguished from *Alternaria radicina* Meier, Drechsl. & Eddy, and the same fungus developed profusely on the affected seedlings and on practically all of the seeds incubated, as well as on celery debris mixed with the seeds. Pycnidia of *Septoria* appeared on a few of the seeds but *Phoma apiicola* was not seen. A few of the less badly affected seedlings produced a number of healthy rootlets in the Petri dishes, and the nurseryman reported that affected seedlings often made fresh roots and recovered from the disease after they had been pricked out.

Alternaria radicina has been known abroad since 1918 as the cause of a Black Rot of carrots, and in recent years it has been found on several occasions attacking carrots in this country (Moore, 1943, p. 44). As far as I am aware, however, it has not previously been reported on celery, although Neergaard (1935, 1936, 1937) has described it as the cause of damping-off of celeriac seedlings in Denmark and of a black mould scab (Selleriens Sortskimmelskurv) of celeriac tubers, on which it forms a dry, brown, cracked crust a few millimetres thick. Neergaard proved that the fungus was pathogenic to the roots, tubers, stems, petioles, leaves, umbels and seeds of celeriac and demonstrated that it could be both soil and seed transmitted. He also found the fungus attacking parsnip roots and parsley in Denmark.

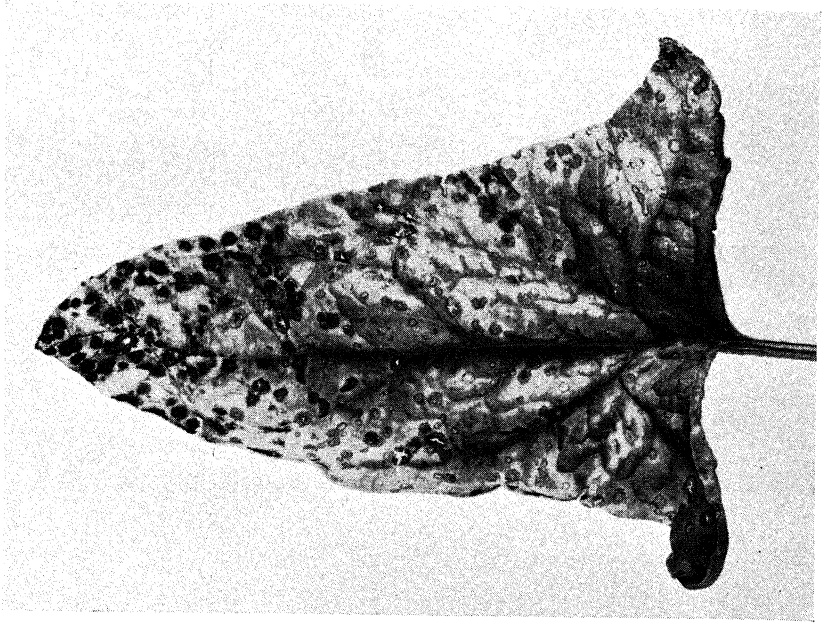
22. Snow rot of wheat (*Typhula graminum* Karst.)

In certain parts of Europe, notably Scandinavia and northern Germany, and in the United States of America and Japan, considerable rotting and yellowing is frequently revealed in winter crops of wheat, barley, and rye when the snow melts in spring. The young plants are backward in growth, the leaves become yellow and brown from the tips downwards, and many plants may be killed outright. Others survive, slowly grow away from the disease, and may produce weak and badly filled heads. The rot is usually attributed to the sclerotium-forming fungus *Typhula graminum* Karst. but, as Remsburg and Hungerford (1933) have indicated, there are probably a number of different species of the form genus *Sclerotium* which attack cereals and grasses. One of them, *S. rhizodes* Auersw., was recorded in England on grasses some years ago (Stirrup, 1932) and another, which corresponded closely to the description of *Typhula graminum* Karst. in its sclerotial state, was found on wheat at Leverstock Green, Herts, in February 1945. Shortly after a heavy fall of snow small patches of the young plants on the headlands of a large field of wheat attracted attention because the outer leaves had begun to turn yellow and wither. When Dr Alex Smith examined some of the plants in the laboratory he found a few small, brown or greyish-brown sclerotia, about the size of a pin's head, on the crowns and the withered leaf sheaths. The symptoms and sclerotia recalled the *Typhula* disease described and illustrated, for instance, by Volk (1937). Only a little material was available and when the field was revisited towards the end of March the wheat was growing vigorously, and, apart from a certain amount of wireworm damage, appeared to be healthy. It was only after careful search in the particular part of the headlands where the patches had been seen, that two or three plants bearing sclerotia

Fig. 1.

Photos by W. F. Buck

Fig. 2.



Photos by W. F. Buck

Fig. 4.

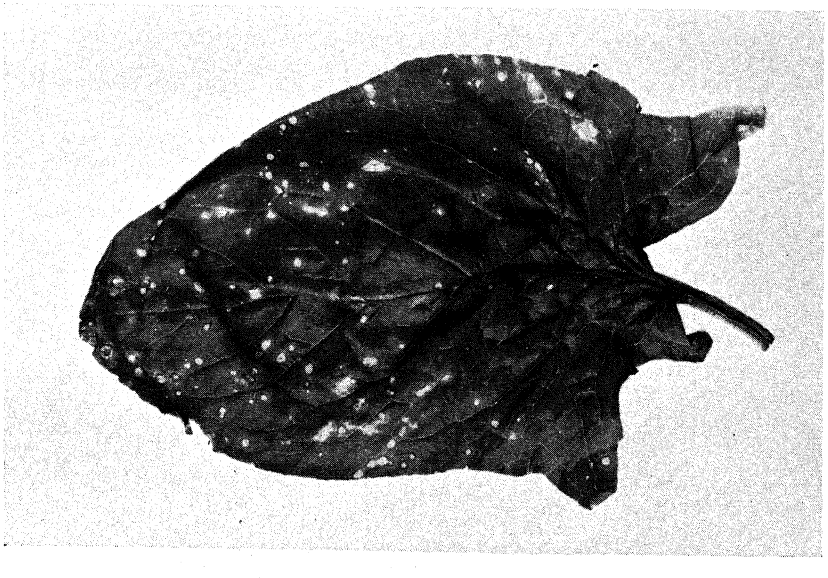


Fig. 3.

could be found. They were present on the oldest withered leaf sheaths, and beyond the fact that they were somewhat dwarfed, the plants appeared to be healthy. Pure cultures were obtained from the sclerotia, and though no sporing stage was observed on the plants or in culture, there is little doubt that the fungus concerned was the one commonly called *T. graminum* Karst. According to Imai (1936), however, it is really distinct from that species and might properly be called *T. itoeana* Imai. In tubes of dextrose agar the fungus produced only a very scanty aerial mycelium and soon began to form one or more coralloid masses of sclerotia on the surface of the agar slope. These masses, which were a few millimetres to over a centimetre across, and projected up to ½ cm. above the agar slope, were honey coloured or pinkish at first, but soon became reddish brown with a rather glistening appearance. In section they consisted of a mass of interlacing, colourless hyphae surrounded by a very narrow but clearly defined, reddish brown rind.

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EXPLANATION OF PLATES VI AND VII

Plate VI

Fig. 1. Tulip bulbs showing scale spotting of uncertain origin.

Fig. 2. Green walnut fruits with ring rot. The one on the right bears sporodochia of *Fusarium* sp.

Plate VII

Fig. 3. Spinach leaf illustrating early stage of attack by *Heterosporium variabile* Cooke.

Fig. 4. A later stage with *Heterosporium* developing on the spots.

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PHYSIOLOGIC RACES OF THE CROWN GALL ORGANISM IN BRITAIN

By H. WORMALD, *East Malling Research Station, Kent*

(With Plates VIII and IX)

The discovery by Smith and Townsend (1907), that crown gall of plants is caused by a bacterium, stimulated research on various aspects of this disease in many countries. Since 1907 hundreds of papers on crown gall have been published, many of them in the United States, particularly by Erwin F. Smith and his school, and by Riker and his collaborators who have given special attention to galls, hairy root, and wound overgrowths of nursery fruit trees (see, for example, Riker & Keitt, 1925, 1926; and Riker & Muncie, 1926). On the Continent, too, many papers have appeared including several dealing chiefly with crown gall on nursery trees, e.g. Stapp (1938), Stapp *et al.* (1938).

In Britain, crown gall has received comparatively little attention, particularly experimentally. Massee (1910*a*, *b*), in early accounts of its occurrence in this country, at first followed Toumey (1900) in attributing the disease to a myxomycete, *Dendrophagus globosus* Toumey, but later (1915) accepted the findings of Smith and Townsend. An anonymous note in the *Journal of the Ministry of Agriculture* in 1911 describes root tumours on sugar beet that were probably crown galls. In 1921 Walkden announced the isolation of *Bacterium tumefaciens* E. F. Sm. & Towns. from a crown gall on Paris daisy (the garden marguerite *Chrysanthemum frutescens*), and was the first to show that the crown-gall organism was actually present in Britain; later Robinson and Walkden (1923) published results of further work on the disease. Ramsbottom (1927) briefly discussed the analogies between plant crown galls and human cancer, with special reference to a paper by Robinson (1927). Berridge (1929, 1930) studied *Bacterium tumefaciens* in relation to the *p*H of cell sap. Butler discussed the anatomy of crown gall in two papers (1930, 1931), the latter one criticizing the emphasis that some workers placed on the analogies between crown galls and animal tumours (including human cancers). The plant disease known as leafy gall, but caused by a different organism, *Corynebacterium fascians* (Tilf.) Dowson, has been investigated in England by Williams (1933–5), and by Lacey (1936*a*, *b*, 1939, 1942).

About the year 1920, crown gall began to be studied at the East Malling Research Station because of its frequent occurrence on apple and pear varieties raised vegetatively (by layering or stooling) for rootstocks. A brief account of trials for its control in the nursery was given by Wormald and Grubb (1922), who later (1924) described field observations with particular reference to the relative susceptibility of the rootstock varieties. When it was found that crown gall caused little or no damage to either apple rootstocks in the nursery or to orchard trees, investigations on an intensive

scale were discontinued, though observations and experiments were made from time to time; Hatton, Wormald and Witt (1926, 1928) described burr-knots in relation to their supposed resemblance to crown gall; Harris (1931) made experimental studies of the relative susceptibility of apple rootstock varieties, and this work was carried a step further by Harris and Pearse (1938) who also induced galls on apple stocks by using a growth-promoting substance. Wormald and Garner (1945) describe an experiment in which an organic mercurial preparation gave noticeable control of crown gall on vegetatively raised apple stocks.

The evidence as to whether crown gall is really detrimental, particularly to apple rootstocks and the trees worked on them, is contradictory. Certain American workers maintain that the galls do have an adverse effect. Fracker (1918*a*, *b*) found that more non-infected trees could be classed as grade I than those bearing galls or hairy root, and Greene and Melhus (1919) write: 'The data presented show that crown gall greatly retards the growth activities of young apple trees. It is followed by a reduction in the amount of increase in trunk diameter, and in the number of twigs, their length, their thickness, and their weight.'

It has been observed at East Malling that, on some batches of comparable trees, the largest galls were on the most vigorous trees, and Russian observers have stated that trees with crown gall are sometimes more vigorous than those free from galls (see Jaczewski, 1935, p. 482). Harris and Pearse (1938) obtained evidence that 'inoculations with *B. tumefaciens* had a measurable stimulating effect on the growth of the host trees'.

Apple trees on vegetatively raised rootstocks planted out at East Malling, some with galls others without, showed no difference in vigour or cropping for eighteen years. It is to be noted, however, that apple rootstocks raised from layers or stools do not become infected until after they have been removed from the 'parent' layer or stool and planted out in the nursery. Seedling apple trees, on the other hand, may develop galls at the collar in their first year (Pl. VIII, fig. 3) and two-year-old seedling apple trees have been seen with galls weighing about 40 % of the total weight of the trees (Wormald & Grubb, 1924): the effect of such large galls relative to the trees bearing them has not been tested.

The galls are sometimes enormous in comparison with the organs bearing them. Apple roots only about 2 mm. in diameter have been found with galls 5–7 cm. across. Such galls must absorb large quantities of nutrients that might have been utilized by the plant for building up normal healthy tissues.

Evidence that crown gall may sometimes be definitely harmful has been seen on pear layers and on raspberry stools where buds have developed into galls instead of into shoots, and on cultivated blackberries where buds on the canes are transformed into galls that sometimes cause the canes to split into strips.

The host plants of crown gall are many; Jaczewski (1935, p. 458) listed 66 species of plants (of thirty-nine different families) on which crown gall had been recorded or had been induced by inoculation up to 1931, and since that time the number has increased considerably.

In connexion with the occurrence of the disease at East Malling the writer collected tumours (from their general appearance and absence of any obvious parasite apparently of the crown gall type) from apple (layered stocks and seedling trees), pear (layered stocks and stools), *Pyrus Toringa* (crown), hawthorn (stools), peach (root), plum (root), loganberry (roots), cultivated blackberry (canes), rose (particularly Manetti root-stocks), hop (crown), beetroot (crown), mangold (crown), melon (leaves), *Scolymus* (crown), dahlia (crown), phlox (stems), hollyhock (crown), *Schizanthus* (crown), runner bean (crown), dock (crown), *Chaerophyllum bulbosum* (crown), *Eucalyptus* sp. (on crown of seedling plant), and lupin (crown). Other plants on which crown gall was found in England during the period 1933–42 (Moore, 1943) are swede, sugar beet, tomato, vegetable marrow, Japanese quince (*Cydonia japonica*, on imported plants), gooseberry (twigs), youngberry, *Asparagus Sprengeri*, and chrysanthemum. The hosts of crown gall recorded for Scotland (Dennis & Foister, 1942) are sugar beet, mangold, tomato, raspberry, *Chrysanthemum indicum*, sweet pea, and *Potentilla* sp. (Gibson's Scarlet).

It is not certain that all the galls examined or recorded were true crown gall caused by *Bacterium tumefaciens*, as superficial examination is not enough for diagnosis, and the isolation of a gall-inducing bacterium having certain cultural characters is the only reliable criterion. Up to the present, Walkden's paper is the only published account of the isolation of a gall-producing bacterium from crown galls (excluding 'leafy galls') found in Britain, though it has been briefly stated that strains of *B. tumefaciens* have been isolated at East Malling (*Rep. E. Malling Res. Sta.* for 1939, p. 28, and 1940, p. 23). It is assumed, however, that *B. tumefaciens* is the cause of these crown galls in Britain (see Moore, 1943, and *Advisory Leaflet*, no. 253, of the Ministry of Agriculture), the assumption being based on the analogy they bear to galls, that have been shown by isolation and inoculation experiments to contain *B. tumefaciens*, on host plants in America and on the Continent. It is to be noted that much of the earlier experimental work in Europe was made with cultures distributed by E. F. Smith, but more recently Stapp and his colleagues in Germany have obtained isolates of *B. tumefaciens* from galls on a number of hosts.

Isolates were obtained from many of the galls I examined, and some of the organisms have proved to be able to induce galls when inoculated into certain plants. Others have given no evidence that they are pathogenic; whether these were 'avirulent' strains of *B. tumefaciens* or quite different organisms was not determined. As the work proceeded it became evident that the pathogenic strains were not identical, and certain differences among them, particularly with regard to their host relationship, are here described.

Isolation

Because of the relatively few bacteria in galled tissues the isolation of the causal organism offers special difficulties, and some workers have had recourse to original methods or special culture media. The method I have generally employed is as follows:

The gall is scrubbed with a hard toothbrush in a stream of tap water and the surface then dried with filter paper. A small piece of the gall is broken off and (impaled on a needle) flooded with a drop of absolute alcohol which is immediately burnt off; a clean surface is cut with a sterile scalpel and a small bit of clean-looking tissue is removed on the point of the scalpel and placed in a sterile petri dish, where it is crushed with the end of a sterile glass rod and then transferred to a tube of carrot extract. Several tubes are prepared in this way and incubated (25° C.). When any tube shows characteristic growth isolation plates of carrot agar are prepared. The features looked for in these preliminary tubes are, slow growth (the liquid becoming slightly turbid only after several days), followed by the formation of a thick, white, somewhat gelatinous pellicle, without any pronounced turbidity of the liquid. The plate colonies are raised, shiny, translucent, with a dense, white centre.

Carrot extract has been found to be a useful culture medium, for the crown gall organism grows well in it and (with transfers every six months) retains its virulence for years without reisolation from a host plant. Thus one of the strains used is that isolated in 1919 by Walkden, who kindly sent me a subculture soon after he described his isolate (Walkden, 1921); it has been grown in carrot extract ever since, and when recently (1943) tested for pathogenicity it produced galls 2–3·5 cm. in diameter on Paris daisy—more than twenty years after its isolation.

Carrot extract agar has been used for the isolation plates, and for streak cultures, in tubes or in plates, for plant inoculations.

The first isolates obtained at East Malling were from a number of apple galls, from a specimen showing hairy root, and from a pear rootstock, but they gave no very conclusive results when inoculated into young apple trees or into stems of tomato, raspberry, *Chrysanthemum coronarium* or *C. frutescens*, although certain apple isolates when inoculated into young apple stems did cause the development of small galls (see Pl. IX, figs. 8, 9), the largest measuring 14 × 8 × 7 mm. and 13 × 12 × 8 mm., and from two of them the organism was reisolated. Patel (1927), and Stapp *et al.* (1938) found that isolates from apple galls are often avirulent, and Brown and Leonard (1932) refer to the frequent occurrence of non-virulent colonies of *Bacterium tumefaciens* on plates made from known crown gall tissue.

On the other hand isolates from other hosts will readily infect apple, and at East Malling large galls have been induced on apple by inoculations with strains from raspberry and from dock (Pl. IX, fig. 10). Walkden's strain, though very virulent on Paris daisy, when inoculated into apple has produced only one small gall from twenty-five inoculations.

Inoculation experiments

During the period 1920–33 many inoculations were made and the results are summarized in Table 1. Some of the strains used were obtained from other workers and their source is given. The general result, usually of a number of experiments, is indicated by + + (well-developed galls), + (small galls), ? (slight swelling, doubtfully gall tissue, possibly wound-callus), 0 (no outgrowth).

Table 1. *Summary of results of inoculations with isolates of* Bacterium tumefaciens, 1920–33

Origin of isolates	Inoculated host plants: Apple	*Chrysanthemum frutescens*	*C. coronarium*	*C. sinense*	Raspberry	Tomato
Apple	+, ?, or o	o	o	—	—	o
Apple (Riker)	—	o	—	—	—	++
Bramble	o	—	—	—	—	—
Daisy (Walkden)	+ or o	++	—	+	+	+
Daisy (E. F. Smith)	—	++	—	—	—	—
Dock	++	o	—	o	++	++
Dock R	—	o	—	—	—	—
Hollyhock	—	—	o	—	—	+
Hop (E. F. Smith)	—	—	++	—	—	++
Poplar (E. F. Smith)	—	—	++	—	—	++
Raspberry 1	++	o	o	o	++	++
Raspberry 2	++	+ or o*	—	?	++	—
Raspberry R	—	o	—	—	—	—
Raspberry (wild)	o	—	—	—	—	—
Raspberry (Riker)	—	o	—	—	—	++
Schizanthus	—	o	—	—	—	?

* Three out of ten inoculations yielded small galls.

The inoculations on Paris daisy and on tomato were, as a rule, well marked; with any one strain the inoculations usually gave either all positive results (with well-developed galls) or all negative (with no trace of galls). On apple, however, with strains virulent for that host some only (25–50 %) of the inoculations were successful, resulting in large galls, the others giving no abnormal growth whatever. Thus in one experiment, using raspberry, dock and daisy (Walkden) strains, each inoculated into sixteen trees, four inoculated with raspberry and five with dock strains yielded large galls, one with daisy strain a small gall, and all the rest (including sixteen inoculated with an apple isolate) gave no abnormal response. In another experiment with raspberry strain on twenty-four apple trees all of the same age and variety, thirteen trees developed large galls, the rest no trace of galls. Similar erratic results have occurred in other experiments on apples, and at present the cause is unknown. Harris and Pearse (1938) have indicated the possibility, however, that the organism may have an appreciable effect on the tree in the absence of a gall, so that failure to produce a gall cannot be accepted as absolute proof of a negative result of inoculation.

That there are races of *B. tumefaciens* which are pathogenic on some hosts and not on others has been established by workers abroad, e.g. Smith, Brown and Townsend (1911), Siegler (1928), Stapp *et al.* (1938), Dame (1938). On the other hand, Kapshuk (1933), in Russia, made several isolations from various hosts and from the soil of infected orchards and found that they were identical in morphological and cultural characters and were equally virulent on beet, tobacco, and tomato.

As shown above certain strains of *B. tumefaciens* readily induced galls on Paris daisy, while others failed on daisy but infected tomato. As this indicated some degree of physiologic specialization, confirmation was deemed necessary. The inoculation experiments, discontinued in 1933,

were therefore resumed in 1939, when some of the strains of previous experiments, and others more recently isolated, were used on Paris daisy and tomato. The results are shown in Table 2.

All the strains used had been isolated by myself except Walkden's daisy strain. The fraction shows the number of inoculations (denominator) and the number yielding galls (numerator). The inoculations were made by placing bacterial slime (from a stroke culture on carrot agar) on the stem and making a puncture through it into the stem, with a sterile needle. The figures in brackets are not strictly comparable with the rest; they show the mean size of the galls that developed, ignoring the inoculations that failed. The Paris daisy plants were from a clone propagated by cuttings; the tomato plants were seedlings of one variety.

Table 2. *Results of inoculations on Paris daisy and tomato*

Strain of *B. tumefaciens*	Paris daisy		Tomato	
	Result	Mean diam. of galls in mm.	Result	Mean diam. of galls in mm.
Daisy	8/8	20·7	6/6	6·3
Dock R*	0/6	—	4/4	15·5
Raspberry R†	0/5	—	4/4	11·0
Bramble	0/4	—	2/6	(2·5)
Blackberry‡	0/4	—	6/6	2·3
Loganberry 1	0/4	—	4/6	(3·2)
Loganberry 2	0/5	—	6/6	10·6
Hollyhock	0/3	—	6/6	7·3
Runner Bean	0/4	—	3/6	(3·3)
Schizanthus	0/4	—	2/4	(3·0)
Control	0/6	—	0/4	—

* Originally from dock but reisolated from tomato.
† Originally from raspberry but reisolated from tomato.
‡ From a cane gall of the cultivated variety Black Diamond.

The table shows that (1) on daisy, only the daisy isolate produced galls, (2) on tomato all produced galls but in various degrees, (3) the largest galls on tomato were induced by isolates dock R, raspberry R, and loganberry 2, those of the daisy strain being only of medium size, (4) one loganberry strain was far more virulent than the other on tomato, (5) an isolate, originally from a weed (dock), and one from a hedgerow plant (bramble) induced galls on tomato, that from dock being particularly virulent.

At each point of inoculation on daisy with the daisy strain, the gall had a double structure; a gall arose at the point of inoculation and another on the opposite side of the stem; these two eventually met so that the stem became girdled.* From each of two inoculations, in addition to the ordinary rounded galls, an elongated gall developed on a petiole immediately above the point of inoculation, on one of them extending along the midrib of the lamina nearly to the apex. Such secondary galls on leaves have been observed by Smith *et al.* (1912), and by Robinson and Walkden (1923).

On the tomato stems a large gall was usually accompanied, on the side opposite the puncture, by a smaller and smoother secondary gall, as in

* In the table each double gall is counted as one.

daisy. On some again there were pimple-like swellings between and around the galls; they were smooth, circular, mostly 2–3 mm. in diameter, and they appeared on all plants inoculated with isolates dock R, raspberry R and loganberry 2, and on one with hollyhock. These small outgrowths appear to be the 'extruding secondary tumors' described by Suit and Eardley (1935).

The original dock isolate was obtained from a gall at the crown of a plant of *Rumex crispus* growing as a weed on the East Malling Research Station. That crown gall occurs on wild plants and weeds is of some significance, since such plants may serve as unsuspected hosts of *Bacterium tumefaciens*. Other workers record its occurrence on *Rumex crispus* (Muncie, 1930), *Rumex acetosella* (Palm, 1934), and *Malva rotundifolia* (Hildebrand & Massey, 1942).

In 1940 similar series of inoculations were made on Paris daisy and tomato, and the original isolates from dock and raspberry were also included. The results were comparable with and confirm those of 1939. Again only the daisy strain infected daisy, with galls 3–5 cm. in diameter. On tomato the largest galls were induced by the original dock and raspberry isolates, those next in size (but only slightly smaller) by dock R, raspberry R, and loganberry 2; the daisy strain again produced rather small galls, and in this experiment isolate loganberry 1 produced no galls.

The results on *Chrysanthemum frutescens* and *C. sinense* suggested that the daisy strain might have some physiological adaptation to plants of the family Compositae, and this was supported by inoculations on stems of the scentless mayweed (*Matricaria inodora*) growing as a weed on the Station, using daisy and dock R strains; the daisy strain produced galls 4–9 mm. in diameter, while no galls appeared on those stems inoculated with dock R, or on control stems. Other plants of the family have not been tested.

In 1941 further experiments were made to test the virulence of certain isolates; the chief points of interest arising from the results were these:

(1) Two fresh isolates had been obtained from raspberry galls in 1940, one from a typical crown gall at the base of a cane, the other from a gall on the upper side of a rhizome; the latter had apparently grown from a bud which had developed into a gall instead of into a normal cane. When inoculated on tomato both isolates produced galls up to 4 cm. in diameter but they were non-virulent on Paris daisy, while Walkden's strain tested on daisy at the same time produced galls 2–4 cm. in diameter. These and previous results showed that three separate isolates from raspberry infected tomato but not Paris daisy; one other produced a few small galls on daisy (raspberry 2, see p. 138).

(2) The original raspberry isolate was a typical smooth type for some years (it was isolated in 1926), but about 1937 it had become 'rough', and stroke cultures on agar were furrowed, dull, and of feeble growth. It was thought that it might have lost its virulence, but when inoculated into tomato stems, through punctures and on the exposed surfaces of cut ends of stems, galls 1·5 cm. in diameter developed at the punctures, and 2 cm. at the cut ends.

(3) The isolate raspberry R (reisolated from tomato in 1927) had been

inoculated into apple stems in 1939, and large galls were present on some of the trees in the winter 1940–1. Isolates, which appeared to be *Bacterium tumefaciens* were obtained from two of these galls, and one of them was inoculated into tomato stems; only very small outgrowths, possibly incipient galls, appeared at some of the punctures. This result suggests that the organism, in passing through apple, had almost lost its virulence. As already mentioned many isolates from galls arising naturally on apple are non-virulent.

In 1942 inoculations with strains isolated from *Rubus* spp. were made on raspberry canes grown in pots in a greenhouse with the results shown in Table 3.

Table 3. *Isolates from* Rubus *spp. inoculated into raspberry canes*

Source of strain	Result	Mean size of galls in mm.
Blackberry (cultivated)	4/12	(2·2)
Bramble	8/12	(2·1)
Loganberry 1	3/12	(2·0)
Loganberry 2	12/12	9·2
Raspberry (crown, 1940)	12/12	8·0
Raspberry (rhizome, 1940)	12/12	9·5
Control	0/15	—

The results are comparable with those on tomato, and show the marked virulence on raspberry of isolates from raspberry and of loganberry 2. The two loganberry isolates were from material (roots) obtained from widely separated places, and they appear, from their different virulence and behaviour in culture (see p. 143) to be different strains.

Culture tests

Although complete cultural examination of the strains used in the inoculation experiments has not been made, the tests applied have shown that the organisms have affinity with certain named strains (Walkden's daisy, and isolates from E. F. Smith and A. J. Riker). The apple and pear strains show these characters in common with named strains:

(1) Gelatin is not liquefied.

(2) Growth is strictly aerobic; there is no growth in the closed arm of fermentation tubes.

(3) Growth in liquid media is almost entirely at or near the upper surface, where a firm pellicle is formed. This pellicle in vegetable extracts is a character common to all strains; in carrot extract, with some isolates it attains a thickness of about 1 cm.

(4) The colonies on carrot agar plates, and the streak cultures, are smooth and glistening, somewhat hyaline but with a dense, more opaque, white centre.

(5) On sterilized potato there is a white, spreading, glistening growth.

In some characters they diverge somewhat from the named strains. The growth in sugar-containing media is very similar but they give a more acid reaction. This production of acid is well shown in milk cultures; with

the apple and pear isolates an acid curd is produced, with the named isolates a soft curd with no acid. In litmus milk there is reduction of the litmus, and the acid reaction is more clearly shown by 'purple milk' (bromo-cresol-purple as indicator) in which the East Malling apple and pear isolates turn the medium yellow, most of the named strains bluish purple. In purple lactose agar the same acid reaction is shown. This production of acid they have in common with Dr Riker's raspberry strain, which however does not show it so markedly as the East Malling apple and pear isolates; this raspberry strain is probably the isolate described by Riker (1923) as turning litmus milk pink.

When it was found that some isolates showed marked virulence on tomato, apple, and raspberry, but not on daisy, cultural tests were made for comparing them with the daisy strain, to ascertain if there was any correlation between cultural reaction and degree of virulence in relation to host plants.

The ten isolates of Table 3 together with the original dock and raspberry isolates were tested on purple lactose agar and indicator milk, for these media had already shown the divergence of the apple isolates from type strains.

On purple lactose (1 %) agar (slope cultures) all gave a slight alkaline reaction within twenty-four hours. Isolates loganberry 1, runner bean, and *Schizanthus* remained alkaline, the rest showed, sooner or later, some acidity, the reaction starting at the upper end of the slope. In isolates dock, dock R, raspberry, raspberry R, bramble and loganberry 2 this acid reaction started on the second day after inoculation and was more pronounced than in the other isolates. It became reversed, however, and after three weeks all again showed an alkaline reaction which persisted to the end of the experiment (three months).

On purple lactose (2 %) agar the reaction was again alkaline within twenty-four hours, but afterwards the acid reaction was more pronounced than with 1 % lactose. Isolates dock, dock R, raspberry, raspberry R, and loganberry 2 showed marked acidity (the medium turning bright yellow throughout) within four to six days, the bramble isolate a little later; this marked acidity persisted (three months). The *Schizanthus* isolate remained alkaline throughout the experiment. The daisy, loganberry 1, bean, blackberry, and hollyhock isolates, after the initial change to alkalinity, gave a slight acid reaction which persisted in the first three, but in the other two reverted to alkalinity.

These results show that the strains most virulent on tomato are those that produce acid most readily from lactose. The bramble strain (feebly parasitic on tomato) also gave an acid reaction on purple lactose agar, but this strain in carrot extract produces more turbidity and a thinner pellicle than the rest, with a tendency to form bubbles of gas at the surface; probably therefore it should not strictly be included under *Bacterium tumefaciens*.

The milk media were (1) plain milk (without indicator), (2) litmus milk, (3) methylene-blue milk, (4) purple milk.

The results as regards curdling of the casein was (as expected) approxi-

mately the same for any one isolate in all four media, the only differences being those of colour due to the indicators.

As the litmus was reduced by all the isolates it proved unsuitable for showing change of reaction.

The methylene blue was reduced by all within four days, but later colour returned in those tubes, which, as shown by the purple milk, were definitely acid.

The indicator of the purple milk was not reduced and so served to show clearly any change in reaction. Within the first four days after inoculation all showed a reaction in the alkaline direction. Later, however, some of the isolates showed a distinct acid reaction, accompanied by curdling of the casein. After five weeks the results were as shown below.

Table 4. *Reaction in purple milk of various isolates after five weeks*

Isolate	Action on casein	Colour of medium	*p*H
Daisy	No curd	Blue purple	7·0
Dock R	Solid curd	Yellow	5·4
Raspberry R	,, ,,	,,	5·4
Bramble	Soft curd with a little whey	Slightly bluer than control	6·3
Blackberry	Slight separation of curd above	Blue purple	7·0
Loganberry 1	,, ,, ,, ,,	Bluer than control	6·6
Loganberry 2	Solid curd	Yellow	5·7
Hollyhock	No curd	Blue purple	7·0
Runner Bean	Slight separation of curd above	Bluer than control	6·5
Schizanthus	,, ,, ,, ,,	,, ,, ,,	6·5
Dock (orig.)	Solid curd	Yellow	5·4
Raspberry (orig.)	,, ,,	,,	5·4
Control	No curd	Purple	6·2

The most striking results from these cultural experiments is that strains non-virulent on chrysanthemum yet most virulent on tomato and apple produced a definite acid reaction (as shown by the final *p*H of the media) in milk and in lactose agar, media containing lactose. What bearing, if any, this has on the relation between these parasites and their hosts has not been investigated.

The general conclusion to be drawn from the inoculations and cultural tests is that within the species *Bacterium tumefaciens* there are, in Britain, physiologic races with different host relationships and habit in cultures.

Four categories can be distinguished:

(1) The daisy (chrysanthemum) type (apparently similar to the original *Bacterium tumefaciens* E. F. Sm. & Towns.) which readily infects Paris daisy; it produces galls of medium size on tomato and raspberry, rarely on apple. This type shows no definite acid action in media containing lactose.

(2) The dock type (including raspberry and probably loganberry 2 isolates) does not infect Paris daisy but produces large galls on tomato, apple, and raspberry. It gives a marked acid reaction in lactose media (an acid curd in milk).

(3) Other isolates do not infect Paris daisy, but induce small galls on tomato; they mostly produce a little acid in purple lactose agar but not in milk.

(4) Isolates from apple and pear have produced either only small galls

or no galls at all on apple stems, and negative results on other hosts inoculated. They develop acid in media containing sugar (sucrose, dextrose, or lactose), and an acid curd in milk cultures.

Summary

Previous descriptions and work on crown gall in Britain are reviewed.

Investigations on crown gall at the East Malling Research Station were started in connexion with its occurrence on apple rootstocks, but gall-inducing isolates were obtained from a number of other hosts for comparison.

The organisms examined showed differences in their host relations and reactions in culture, and four categories can be distinguished.

Of two of the organisms isolated one was from a common weed (curled dock) and the other from a hedgerow plant (bramble).

For cultures of *Bacterium tumefaciens* isolated by other workers I am indebted to the late Dr Erwin F. Smith, to Dr A. J. Riker, and to Mr H. Walkden. I thank also Mr R. V. Harris for his interest and help throughout the investigation and for helpful criticism of the script of this paper.

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EXPLANATION OF PLATES VIII AND IX

PLATE VIII. Natural infections

Fig. 1. Crown gall on dock (*Rumex crispus*).
Fig. 2. Crown gall on raspberry.
Fig. 3. Galls on seedling apple trees.
Fig. 4. Basal, lateral and root galls on an apple rootstock.

PLATE IX. Galls induced by inoculating with pure cultures of *Bacterium tumefaciens*

Fig. 5. Tomato stem inoculated with isolate from dock.
Fig. 6. Tomato stem inoculated with isolate from loganberry.
Fig. 7. Tomato stem inoculated with isolate from daisy (*Chrysanthemum frutescens*).
Figs. 8, 9. Apple stems inoculated with isolates from apple.
Fig. 10. Apple stem inoculated with isolate from dock.

All reduced to $\frac{3}{4}$ natural size.

(*Accepted for publication* 18 *July* 1945)

Fig. 1.

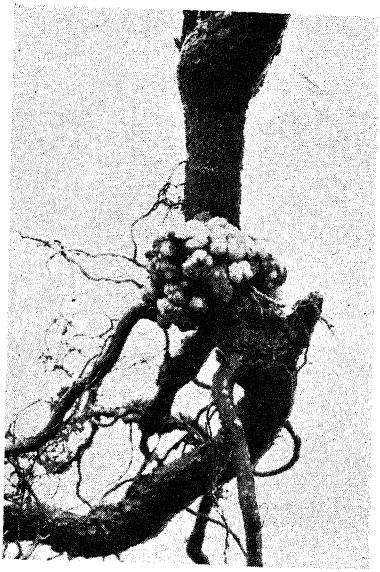
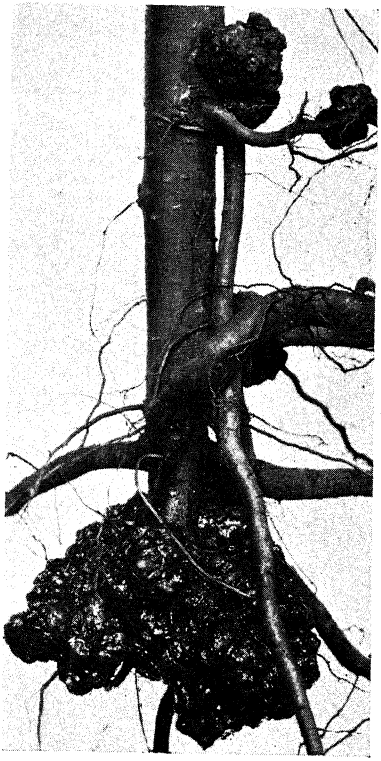


Fig. 2.



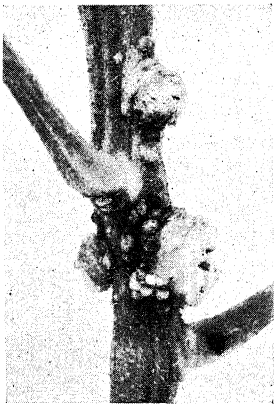


Fig. 5.

Fig. 6.

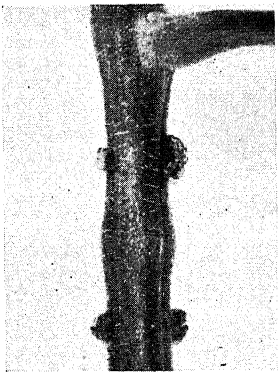


Fig. 7.

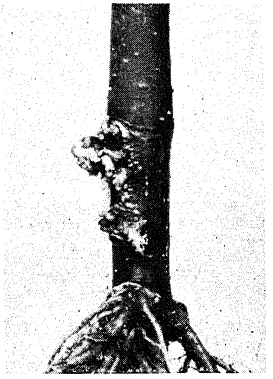


Fig. 8.

PLANT PATHOLOGY MEETING, EAST MALLING

20–21 July 1945

The Nineteenth Annual Plant Pathology Field Day and the annual phytopathological meeting were combined as a two-day meeting which was held at the East Malling Research Station, by courtesy of the Director, on 20 and 21 July 1945.

A party of approximately fifty members and friends was officially welcomed by the Director, Dr R. G. Hatton, C.B.E., F.R.S., at Bradbourne House, after which short talks were given by Messrs R. V. Harris, H. Wormald, H. B. S. Montgomery, and M. H. Moore on special problems under investigation at the Station. Lunch was taken at Bradbourne, following which a tour was made of the plantations, and demonstrations were given on Shoot Wilt of Plum Layers (*Cylindrocladium* sp.) by Dr Wormald, Bacterial Canker of Stone Fruits (*Pseudomonas mors-prunorum*) and Apple Canker (*Nectria galligena*) by Mr M. H. Moore, Control of Raspberry Cane Spot (*Elsinoe veneta*) by Dr R. V. Harris, and the recording of Apple Scab (*Venturia inaequalis*) on foliage by Miss M. Bennett. After tea a very interesting series of laboratory exhibits was inspected including the Laboratory Testing of Fungicides by Dr H. Shaw and Miss M. P. English, Species and Strains of *Verticillium* by Mr I. Isaac, Shoot Wilt of Plum Layers and Strawberry Leaf Blotch by Dr Wormald, *Verticillium* Wilt of Hops by Dr W. G. Keyworth, and Virus diseases of Fruit by Mr I. W. Prentice.

The second day was spent mainly on hop problems, and introductory talks were given by Messrs Harris, Keyworth and Isaac. Another tour of the experimental plots followed before returning to an excellent lunch kindly provided by the Station. The afternoon was devoted to visiting hop gardens in the Paddock Wood area, where examples of both 'fluctuating' and 'progressive' types of *Verticillium* outbreaks were inspected. Tea was taken after returning to the Station and the gathering dispersed at 5 p.m.

The meeting was voted a great success by all present, and the credit for this is due entirely to the hard work of the East Malling staff who were responsible for all the arrangements. The meeting expressed its appreciation and thanks to the Director and staff for a most enjoyable and instructive visit and for the very efficient way in which the programme had been carried out.

J. H. Western
Secretary, Plant Pathology Committee

FORAYS

By the Foray Committee

During the period 1897–1939 the Society held sixty-nine major forays devoted to the mycological exploration of localities in the British Isles, namely, five in Scotland, in collaboration with the former Cryptogamic Society of Scotland, four in Ireland, and the remainder in England and Wales. Twenty-seven were spring forays, within the period of 9 May to 10 June, and the remainder autumn forays within the period 5 September to 10 October. On a basis of the collections exhibited on these occasions, supplemented by lists of personal observations supplied by the members present, the Secretary compiled lists of species which were published in the *Transactions*.

There is little doubt that these forays served several useful purposes, as in maintaining a somewhat tenuous 'tradition of species', in introducing members to suitable collecting grounds in districts not personally familiar to them, and, to a rather disappointing extent, in encouraging the study of fungi by local naturalists. The Foray Committee feels, however, that a programme of field work based on a more ecological outlook may be envisaged for the future without losing the advantages of the old forays.

Study of the published lists suggests that the time is ripe to modify the record-taking at forays. For these reasons:

(1) While providing evidence of the occurrence of a fungus in a given district on a given date, the Society's published lists afford little or no clue as to its precise habitat, its relative abundance, or its continuous or discontinuous annual occurrence.

(2) Accurate determination of fungi is notoriously difficult, yet as a rule no indication is afforded of the authority for the published determinations, even of rare or critical species.

(3) No systematic attempt has been made to preserve specimens, or even drawings, of the rarer British species.

The Committee accordingly proposes that:

(1) Each foray should be devoted in part to the collection and examination of the fungus flora of a well-defined type of vegetation. It is hoped in time to cover all the recognized types of woodland and also to visit other types of vegetation, such as moorland, heath, and pasture, each with its characteristic though less abundant fungus flora, which have been much neglected by the Society in the past. It is not proposed to publish separate lists for every foray, but to visit each selected type of vegetation a number of times over a period of years and finally to publish a survey of the fungi found there. With this in view the Committee invites all members present at a foray to co-operate in the more intensive examination of the type of habitat selected, and in the depositing of material of all species collected there in containers provided for the purpose. As has been the practice hitherto, arrangements will be made wherever possible to name and exhibit these collections during the foray. Reports of the year's forays will continue to appear in the *Transactions*, on the lines of those published during the war, but more detailed. They will include observations on collections of special interest and progress reports on the Society's ecological studies.

(2) For the benefit of future workers the referees responsible for the names in published lists should be indicated.

(3) An attempt should be made to preserve representative material of the species recorded and especially of new, rare or critical species, where it will be available for consultation by members or others interested in the validity of a particular record or engaged in monographic study of a species or larger group.

It is hoped in 1946 to revert to the longer spring and autumn forays. The Committee feels, however, that the day forays held at localities easily accessible from London have much to commend them and can be usefully employed to promote a mycological survey of south-eastern England. If this principle is accepted, the longer forays should be devoted to examination of less accessible districts and less-known types of vegetation well outside the London basin.

PROCEEDINGS

Meeting held in the Department of Biology, Chelsea Polytechnic, London, at 11.30 a.m. 17 February 1945. The President, G. Smith, M.Sc., in the Chair.

The dispersal of air-borne fungus spores

P. H. GREGORY. Air-borne spore dispersal [see these *Transactions*, XXVIII, 26–72].
W. A. R. DILLON-WESTON. Observations on spore dispersal.

Discussion to which C. G. Dobbs, C. T. Ingold, E. W. Mason, G. Samuel, and others, contributed.

At the end of the afternoon session the films 'Life Cycle of Pin Mould', 'Potato Blight' and 'Cereal Seed Disinfection' were shown.

Meeting held in the Herbarium of the Royal Botanic Gardens, Kew, at 2.30 p.m. 20 April 1945. The President, G. Smith, M.Sc., in the Chair.

Exhibition of Mycological Books

from the libraries of the Royal Botanic Gardens, Kew, the Imperial Mycological Institute, and Members.

The exhibits, which were commented on by Miss E. M. Wakefield and the Secretary, included illustrated books on the Agaricaceae, nineteenth-century books on plant pathology, the starting-points for international nomenclature, a number of association books, and the Society's collection of local lists.

In addition, there was an exhibit of sclerotia and pseudosclerotia, mostly from the collection of the late Prof. Buller.

CORRECTION

In the paper on 'Some methods for the study of moulds', Fleming and Smith, *Trans. Brit. Myc. Soc.* 1944, XXVII, 13–19, the word 'cellophane' was used inadvertently in such a manner as might create the impression that cellophane is the name of a material. It has been brought to our notice that the word 'Cellophane' is the trade mark of British Cellophane Limited, and denotes exclusively and distinctively the brand of cellulose sheets and films supplied by that Company. The correct designation of the recommended material is regenerated cellulose film, or viscose film.—ED.

REVIEWS

The Gasteromycetes of Australia and New Zealand. By G. H. CUNNINGHAM. (Dunedin, New Zealand, privately printed, 1944.) Pp. xv + 226. 37 black and white plates, 2 text-figures.

The Gasteromycetes present some difficult problems of both taxonomy and nomenclature. The monographic treatments by De Toni, Ed. Fischer, Hollós and others disagree as to the position to be allotted to certain families and genera, and none has seemed entirely satisfactory. Fischer's classification in the second edition of Engler and Prantl's *Natürliche Pflanzenfamilien* in 1933 differs profoundly from that in the first (1900) edition, particularly in the placing of such genera as *Podaxis* and *Secotium*. Attempts to base classification upon developmental studies have not met with much success, and according to Dr Cunningham 'developmental types have little if any value as a basis for taxonomic treatment of genera and higher ranks'. Most modern workers accept the five orders Phallales, Hymenogastrales, Lycoperdales, Sclerodermales and Nidulariales, which are readily recognized groups, but within them families and genera are not so easily defined. One reason for the confusion existing is undoubtedly the difficulty of obtaining adequate material for study. Apart from the large cosmopolitan genera like *Lycoperdon* and *Geaster*, many of these plants are inhabitants of the desert regions of the globe, and of some species only isolated and poorly preserved specimens have reached European herbaria. Fortunately in recent years mycologists in Australasia, in Africa and America, regions where suitable conditions for the growth of Gasteromycetes are to be found, have turned their attention to the group and there is now a good prospect that a better understanding of these plants will come about.

Dr G. H. Cunningham began work on the Gasteromycetes of Australia and New Zealand more than 20 years ago, and his first revisions appeared in 1924. Since then he has produced a long series of papers, published chiefly in the Proc. Linn. Soc. New South Wales and in the Trans. New Zealand Institute, in which he has dealt successively with all the genera of Gasteromycetes found in the region, and has carried out developmental studies of some species. He has now collected together the results of his research work in the present volume, which is not a mere reprint of the earlier papers but a considered revision of the whole group in the light of knowledge so far attained.

Dr Cunningham's book covers Australia, Tasmania, New Zealand and the contiguous small islands, but not the subtropical islands of Fiji, Samoan Islands, New Hebrides, and New Caledonia. This is to be regretted, for one would have liked to know Dr Cunningham's views on, for instance, the genus *Le Ratia*, described from New Caledonia.

In his introductory matter he discusses among other things the cytology of the Gasteromycetes and studies in development. Following Lohwag and Fischer, four types of development are distinguished, namely lacunar, coralloid, pileate, and multipileate, the latter confined to members of the Clathraceae, and the coralloid to one subfamily of the Hymenogastrales. Since the lacunar and pileate types occur in five and three of the orders respectively, and related genera may show different developmental types, Dr Cunningham comes to the conclusion quoted above as to the taxonomic value of developmental studies. Theories as to phylogeny in the group are at present of little value, but no author seems able to resist making guesses; Dr Cunningham is no exception. There is a summary of the known distribution of genera and species, and a good comparative account of the chief systems of classification which have been proposed, culminating with a key to the arrangement of families and genera adopted by the author. He has the usually accepted five main orders, and follows Fischer's 1900 classification in placing the Secotiaceae as a family in the Hymenogastrales. He rejects entirely the suggestion of Agaric affinities for *Secotium* and *Podaxis*. *Mesophellia*, *Castoreum* and *Abstoma* are put together in the tribe Mesophelliae of Lycoperdaceae. *Phellorina* Berk. and *Chlamydopus* Speg. are classed with *Podaxis* in the subfamily Podaxonoideae of Tulostomaceae. It will be seen from these examples that Cunningham favours Fischer's 1900 classification rather than that adopted in 1933. In this account of classification, the author has italicized the names of certain genera and left others in roman type. In a footnote (p. 31) he says that 'valid genera are given in italics'. Presumably he means 'good' genera, not merely names which are

'valid' nomenclaturally, and one is moved to protest against this all too common confusion in the use of the word 'valid'. Even so, it is difficult to understand quite what the author really intends, for the same name is treated differently on different pages. Thus on p. 34 *Cauloglossum* is italicized, while on p. 35 it is not; *Polyplocium* is italicized on p. 35, and not on p. 36, though on both it is placed in Secotiaceae. The greater part of the volume is occupied by descriptions, with keys to both genera and species. Synonymy is given fully. Certain advances on the author's previous papers are noted at once, as that the genus *Bovistella* Morgan is discarded and the species referred to *Bovista*; similarly the author's genus *Phallobata* (1926) is now referred to *Hysterangium*. Dr Cunningham has followed Miss Morse in the treatment of *Podaxis*, so that he now has only one species instead of two.

In nomenclature he has attempted to follow the International Rules, and has therefore rightly adopted *Geastrum* Pers. instead of *Geaster* Fr. It was not, however, necessary to make 'new combinations' under *Geastrum*, since these two names are orthographic variants; nor did Fischer consider this necessary, although Cunningham cites him as having done so. The nomenclature of fornicate Geasters is extraordinarily difficult, but there seems to be no justification for the adoption of *Geastrum minus* (Pers.) Fischer, using a varietal epithet of Persoon's, since the first epithet used for this fungus in the rank of species was *quadrifidum* Nees (1817), which according to Art. 58 should therefore be adopted. There are a few slips and spelling mistakes which should be corrected if another edition is called for. The author must have a liking for the letter *i*, since almost throughout he spells *Phellorina* Berk. as *Phellorinia*, and *Lycogalopsis* as *Lycogaliopsis*! These small errors do not detract, however, from the great value of the book. It is indispensable to students of the Gasteromycetes of any region. The illustrations are from photographs, and are most valuable when of fresh material. Plates 33–37 are of spores and capillitium, the spores all magnified 700 times, so that they are easily compared.

E. M. WAKEFIELD

Root Disease Fungi. By S. D. GARRETT. (Waltham, Mass.: Chronica Botanica Co.; London: Wm. Dawson and Sons, 1944.) 177 pp. 9 figs. $4.50.

Mr Garrett's book is vol. 1 of the *Annales Cryptogamici et Phytopathologici*. It deals in fifteen chapters with a selection of well-known fungi which attack the roots of plants and cause diseases of notable economic importance. The earlier chapters give a general account of the fungi which cause root diseases, with more detailed accounts of those species which the author has chosen for full description; the later chapters deal with the principles of control, mainly in relation to methods of cultivation. Throughout the book, an endeavour is made to emphasize the ecological attitude, and to present fungus, host and environment all as parts of a shifting, living relationship. This is a most welcome attitude, for there can be little doubt that the progress of plant pathology, of mycology, and indeed of botany as a whole, depends much on those practitioners who keep the living organisms in the forefront of their thoughts and work. In intention the book is excellent; regrettably, intention and performance do not match.

The value of Mr Garrett's book is diminished because two separate classes of information have not been kept sufficiently distinct. The presentation lacks in sharpness because the author has been too scrupulous in acknowledging the work of other investigators in a field which he has himself deeply and profitably worked. Acknowledgements, and details of the fungi are so mingled that it is often difficult (and even wearisome) for the reader to get a clear impression either of the fungi or of what has been written about them. The printing of personal names in capital letters, the repetition of the same names in close proximity on the same page, the provision of a long list of references, and then of an index of authors, all these give an impression, no doubt wholly contrary to the intentions of the author, that the book might better have been called *A History of the Investigation of the Root Disease Fungi*; even so, it would have been a chronicle rather than a history.

It is fitting and decent that due acknowledgement should be made when an author uses the work of others. It is common form to make full acknowledgements when a student is preparing a thesis or when an investigator is preparing for publication an account of a detailed piece of work; such writings are provisional in character, and they are not expected to be fully polished. In contrast, a book should have a finished appearance;

it should set out clearly what the author knows and thinks about his chosen subject, and it should not lay on to the reader the task of sorting out for himself the diverse lines of knowledge covered by the book. Mr Garrett's wish to give full recognition to his sources has hindered him from helping his readers as fully as could be desired.

Root Disease Fungi will be a useful source of information to the specialist, but it will not be the everyday companion of the general student. In due time, doubtless, it will engender a more mature and polished account of an interesting and important set of fungi. The knowledge which Mr Garrett has spread out shows that an intelligent use of biological methods of control may well lead to better results than have been obtained by some of the rough and ready methods of the past.

The book is well printed and bound, but the price is rather high for a book containing so few illustrations; that high price is no doubt due to the war.

B. BARNES

An Introduction to the Taxonomy and Nomenclature of Fungi. By G. R. BISBY. (Imperial Mycological Institute, Kew, Surrey, 1945.) 117 pp. 5*s*.

The lack of interest, and even impatience, often shown by naturalists when a discussion arises on the principles of taxonomy and nomenclature is regrettable, but too often those principles are discussed in an atmosphere of mystery which is repellent to workers whose primary interest is in living things. The title of Dr Bisby's book may well mislead, for that title, while fairly indicating the subject of the book, gives no hint of the broad common sense and ripe experience which show on every page that the author has written. Dr Bisby has put into print the things that many of us have said when we have been trying to encourage the young idea, but no one seems before to have included between the covers of one book all that the author has now set down, nor to have given sound guidance in so attractive a form; it is remarkable how dry bones have been made alive. Dr Bisby does not try to play at the now somewhat discredited diversion of debunking, but a reader who knows him can sense the undercurrent of kindly fun, and as he reads can picture the author writing many a sentence with more than a twinkle in his eye.

While it may be that the younger workers in mycology will benefit most from this book, there is much in it that should be of value to the practising botanist, no matter what his special interests may be.

The last fifty pages of the book are occupied by a reprint of the current version of the International Rules of Botanical Nomenclature. These Rules have not been so generally available as could be wished, so that the reprint satisfies a real need. To the reprint, Dr Bisby has added a number of comments and suggestions which provoke thought and may stimulate some necessary action.

B. BARNES

LIST OF MEMBERS

Correct to 30 November 1945

Honorary Members

Lister, Miss Gulielma, F.L.S., 871 High Road, Leytonstone, London, E. 11. (1903.) (1924.)
Maire, René, D.Sc., F.M.L.S., Professeur à la Faculté des Sciences de l'Université, Algiers, Algeria, N. Africa. (1907.) (1939.)
Petch, T., B.A., B.Sc., North Wootton, King's Lynn, Norfolk. (1911.) (1941.)
Rea, Carleton, B.C.L., M.A., 6 Barbourne Terrace, Worcester. (1896.) (1918.)
Wakefield, Miss E. M., M.A., F.L.S., The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1911.) (1941.)

Members

Aberdeen, The University Library. (1916.)
Adams, Rev. J. H., Landulph Rectory, Hatt, Saltash, Cornwall. (1919.)
Ainsworth, G. C., B.Sc., Ph.D., F.L.S., Belmont Laboratories, Ventnor Road, Sutton, Surrey. (1931.)
Alaily, Y. A. S. El, Mycological Section, Cotton Research Board, Giza, Egypt. (1935.)
Alcock, Mrs N. L., M.B.E., F.L.S., 61 Holywell, Oxford. (1919.)
Allchin, J. P., A.I.C., Ministry of Health, Regional Blood Transfusion Service, 17 Highfield Road, Birmingham, 15. (1942.)
Armitage, F. D., A.R.P.S., F.R.M.S., Bargrove Lodge, Boxmoor, Herts. (1942.)
Ashby, S. F., B.Sc., c/o Barclays Bank, Kew Green, Kew, Surrey. (1926.)
Atkins, F. C., Yaxley, Peterborough, Northants. (1945.)
Atkins, R. H., 2 Mersey View, Garston, Liverpool, 19. (1943.)
Bacon, Mrs Alice, B.Sc., F.L.S., Technical College, Brighton, Sussex. (1938.)
Badcock, E. C., Forest Products Research Laboratory, Princes Risborough, Bucks. (1944.)
Baker, R. E. D., B.A., Imperial College of Tropical Agriculture, Trinidad. (1944.)
Balfour-Browne, Mrs F. L., M.Sc., Department of Botany, British Museum (Natural History), Cromwell Road, South Kensington, London, S.W. 7. (1930.)
Barnes, B., D.Sc., Ph.D., Sec.L.S., Chelsea Polytechnic, London, S.W. 3. (1922.)
Barnes, Miss E. M., B.Sc., Bengers Ltd., Holmes Chapel, Cheshire. (1943.)
Barr, Rev. Robert, T.D., M.A., The Manse, Neilston, Renfrewshire. (1918.)
Barrington, Dr F. J. F., 48 Wimpole Street, London, W. 1. (1901.)
Baruah, Hitendra Kumar, M.Sc., The Botany School, Cambridge. (1938.)
Bates, G. R., Ph.D., c/o Agricultural Dept., P.O. Box 387, Salisbury, Southern Rhodesia. (1930.)
Batko, Dr S., The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1944.)
Bawden, F. C., M.A., Rothamsted Experimental Station, Harpenden, Herts. (1941.)
Beaumont, Albert, M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon. (1924.)
Bennett, Miss M., B.Sc. (Hort.), East Malling Research Station, nr. Maidstone, Kent. (1945.)
Bewley, W. F., C.B.E., D.Sc., Experimental and Research Station, Cheshunt, Herts. (1922.)
Biffen, Professor Sir Rowland H., M.A., F.R.S., 136 Huntingdon Road, Cambridge. (1899.)
Biggs, Miss Rosemary, B.Sc., Ph.D., M.B., B.S., The Radcliffe Infirmary, Oxford. (1945.)
Biologist, Plant Research Laboratory, Horticultural Gardens, Burnley, Victoria, Australia. (1921.)
Birmingham Natural History and Philosophical Society, c/o J. W. Moore, F.R.E.S., 151 Middleton Road, King's Norton, Birmingham, 30. (1920.)

Bisby, Guy R., Ph.D., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1921.)
Blackman, Professor V. H., M.A., Sc.D., F.R.S., F.L.S., 17 Berkeley Place, Wimbledon, London, S.W. 19. (1900.)
Blackwell, Miss Elizabeth Marianne, M.Sc., F.L.S., Botanical Department, Royal Holloway College, Englefield Green, Surrey. (1917.)
Blumer, Dr S., 9 Frohmatt Strasse, Waedenswil, Switzerland. (1936.)
Boston, The Mycological Club, The Farlow Laboratory and Herbarium, Divinity Avenue, Cambridge, Mass., U.S.A. (1926.)
Boughey, A. S., B.Sc., Ph.D., Department of Botany, University College, Exeter. (1945.)
Bourgin, Dr Viennot, École Nationale d'Agriculture de Grignon, Seine-et-Oise, France. (1936.)
Braid, Professor K. W., B.A., B.Sc., West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C. 2. (1922.)
Brenchley, G. H., B.A., Clare College, Cambridge. (1925.)
Brett, Miss M., M.Sc., Ph.D., F.L.S., Northern Polytechnic, Holloway Road, London, N. 7. (1921.)
Brian, Dr P. W., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1944.)
Brierley, Professor W. B., D.Sc., F.R.A.I., F.L.S., Department of Agricultural Botany, The University, Reading. (1919.)
British Museum, The Trustees of, Cromwell Road, South Kensington, London, S.W. 7. (1914.)
Broadfoot, Miss J., B.Sc., Plant Pathology Dept., School of Agriculture, Bangor, Caernarvon. (1945.)
Brooks, Professor F. T., M.A., F.R.S., F.L.S., The Botany School, Cambridge. (1907.)
Brown University, The Library, East side Station, Providence, R.I., U.S.A. (1920.)
Brown, Professor W., M.A., D.Sc., F.R.S., Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1922.)
Bruxelles, Jardin Botanique de l'État, c/o M. P. van Aerdschot. (1911.)
Buckley, W. D., 'St Anthony', Leigh Park, Datchet, Bucks. (1916.)
Buddin, Walter, M.A., Laboratory of Plant Pathology, University of Reading, 7 Redlands Road, Reading. (1921.)
Bunting, R. H., F.L.S., 3 Stanton Court, Weymouth. (1921.)
Burges, N. A., The Botany School, Cambridge. (1935.)
Burr, S., M.Sc., Department of Agriculture, The University, Leeds. (1924.)
Caldwell, J., D.Sc., Ph.D., Department of Botany, University College, Exeter. (1932.)
Callen, E. O., 9 Lauderdale Street, Edinburgh, 9. (1941.)
Cambridge, The Botany School. (1920.)
Campbell, A. H., B.Sc., Ph.D., Glaxo Laboratories Ltd., Barnard Castle, Co. Durham. (1934.)
Canter, Mrs P. John, 40 Stavordale Road, Highbury, London, N. 5. (1944.)
Carmichael Medical College, Belgachia Road, Calcutta, India. (1922.)
Carrothers, E. N., L.M.S. Railway, York Road Station, Belfast. (1925.)
Cartwright, K. St G., M.A., F.L.S., The Old Vicarage, Towersey, Thame, Oxon. (1913.)
Cayley, Miss Dorothy M., Foxhall Cottage, Kelshall, nr. Royston, Herts. (1913.)
Chaudhuri, Professor H., M.Sc., Ph.D., University of the Punjab, Lahore, India. (1920.)
Cheal, W. F., St Kenya, Sutton Road, Wisbech, Cambs. (1927.)
Chesters, Professor C. G. C., B.Sc., M.Sc., Ph.D., University College, University Park, Nottingham. (1930.)
Christensen, Dr Clyde M., University Farm, St Paul, 8, Minn., U.S.A. (1945.)
Ciferri, Professor Dr R., Assistant Director, Laboratorio Crittogamico, Casella Postale 165, Pavia, Italy. (1926.)
Clapham, Professor A. R., M.A., Ph.D., Department of Botany, The University, Sheffield. (1931.)

Cleland, J. Burton, M.D., Professor of Pathology, University of Adelaide, South Australia. (1918.)
Clouston, D., M.A., D.Sc., North of Scotland College of Agriculture, 41½ Union Street, Aberdeen. (1931.)
Colhoun, J., B.Sc., Ph.D., Plant Disease Division, Ministry of Agriculture, Queen's University, Belfast, Northern Ireland. (1943.)
Colson, Miss B., B.Sc., Ph.D., Department of Botany, The University, Reading. (1934.)
Cook, W. R.-I., B.Sc., Ph.D., Department of Botany, University College, Newport Road, Cardiff. (1924.)
Cooke, G. J., 143 Newmarket Road, Norwich. (1933.)
Cooper, Mrs V. Astley, South Lodge, Reading Road, Cholsey, Wallingford, Berks. (1921.)
Cornell University, The Library, New York State College of Agriculture, Ithaca, N.Y., U.S.A. (1920.)
Corner, E. J. H., M.A., F.L.S., Assistant Director, Botanic Gardens, Singapore, Straits Settlements. (1924.)
Cotton, Arthur D., O.B.E., F.L.S., Keeper, The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1902.)
Croxall, H. E., B.Sc., Research Station, Long Ashton, Bristol. (1937.)
Curtis, Miss Kathleen M., M.A., D.Sc., D.I.C., F.L.S., Mycologist, Biological Department, Cawthron Institute of Scientific Research, Nelson, New Zealand. (1917.)
Cutting, E. M., M.A., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1920.)
Dade, H. A., A.R.C.S., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1927.)
Dale, W. T., B.Sc., Imperial College of Tropical Agriculture, Trinidad. (1944.)
Davies, D. L. G., Ph.D., Agricultural Buildings, University College of Wales, Aberystwyth. (1938.)
Davies, D. W., B.Sc., Adviser in Mycology, Agricultural Buildings, University College of Wales, Aberystwyth. (1923.)
Davies, H. M., B.Sc., Ph.D., 9 Queen's Road, Richmond, Surrey. (1944.)
Day, W. R., B.A., B.Sc., Imperial Forestry Institute, Oxford. (1928.)
Deacon, Dr G. E., Brundall, Norwich. (1933.)
Dehra Dun, The Forest Botanist, Forest Research Institute and College, U.P., India. (1929.)
Deighton, F. C., M.A., Mycologist, Department of Lands and Forests, Freetown, Sierra Leone, West Africa. (1925.)
Delhi, Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, India. (1921.)
Dennis, R. W. G., Ph.D., The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1932.)
Dickinson, S., Ph.D., School of Agriculture, Cambridge. (1921.)
Dobbs, C. G., B.Sc., Ph.D., Botanical Department, King's College, Strand, London, W.C. 2. (1933.)
Dodge, Dr Carroll W., Missouri Botanical Garden, 2315 Tower Grove Avenue, St Louis, Missouri, U.S.A. (1926.)
d'Oliveira, Professor B., Laboratorio de Patologia Vegetal, Instituto Superior de Agronomia, Tapada da Ajuda, Lisboa, Portugal. (1939.)
Dowson, W. J., M.A., D.Sc., The Botany School, Cambridge. (1920.)
Duncan, J. T., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1930.)
Dunston, Capt. Ambrose E. A., Burltons, Donhead St Mary, Wiltshire (via Shaftesbury). (1937.)
Edinburgh and East of Scotland College of Agriculture, Edinburgh. (1920.)
Elliott, Mrs J. S. Bayliss, D.Sc. (B'ham), B.Sc. (London), Arden Grange, Tanworth-in-Arden, Warwickshire. (1911.)
Ellis, E. A., Castle Museum, Norwich. (1937.)

Ellis, E. H., B.Sc., Gramarye, Farley Green, Guildford, Surrey. (1936.)
Emerson, R., Dept. of Botany, University of California, Berkeley, Calif., U.S.A. (1938.)
English, Miss M. P., East Malling Research Station, nr. Maidstone. (1943.)
Essex Field Club, c/o Mr Percy Thompson, F.L.S., Essex Museum of Natural History, Romford Road, Stratford, London, E. 15. (1919.)
Exeter, Librarian, University College of the South-West of England. (1926.)
Eyre, Miss J. C., Wellbottom Lodge, Moulton, nr. Newmarket. (1915.)
Feilden, G. St Clair, B.A., Imperial Bureau of Horticulture and Plantation Crops, East Malling, nr. Maidstone, Kent. (1944.)
Ferns, J. F., M.Sc., A.I.C., 16 Lawn Crescent, Kew, Surrey. (1942.)
Findlay, W. P. K., D.Sc., A.R.C.S., Forest Products Research Laboratory, Princes Risborough, Bucks. (1928.)
Fisher, S. D. P., Sackville Street, Leeds. (1930.)
Fitzpatrick, Professor H. M., Ph.D., 220 Bryant Avenue, Ithaca, New York, U.S.A. (1935.)
Fletcher, Mrs P. T., B.A., 11 Arundel Court, Jubilee Place, Chelsea, London, S.W. 3. (1935.)
Foister, C. E., B.A., Ph.D., Plant Pathology Service, Seed Testing Station, East Craigs, Edinburgh, 12. (1940.)
Fraser, Miss Lilian R., D.Sc., Biological Branch, Dept. Agriculture, Box 36A, G.P.O., Sydney, Australia. (1938.)
Gadd, C. H., D.Sc., Tea Research Institute, St Coombs, Talawakelle, Ceylon. (1921.)
Gardner, Capt. Frederic, c/o Barclays Bank, Jersey, C.I. (1898.)
Garrett, S. D., M.A., D.I.C., Rothamsted Experimental Station, Harpenden, Herts. (1936.)
Ghamrawy, Ali K., 39 Monirah Street, Cairo, Egypt. (1932.)
Gilbert, E. J., Docteur en Pharmacie, 4 Rue de Musset, Paris (16e), France. (1924.)
Gilbert, Dr E. M., Botanical Department, University of Wisconsin, Madison, Wis., U.S.A. (1922.)
Gill, G. E., LL.B., Law Library, Four Courts, Dublin. (1937.)
Gillespie, J., B.Sc., Department of Botany, The University, Reading. (1938.)
Gilson, Mrs M. R. [née Brown], Newnham College, Cambridge. (1936.)
Glenn, Miss E. M., B.Sc., East Malling Research Station, nr. Maidstone, Kent. (1942.)
Glynne, Miss Mary D., D.Sc., Rothamsted Experimental Station, Harpenden, Herts. (1932.)
Goodman, R. E. J., Norfolk Flax Establishment, Flitcham Abbey, Flitcham, King's Lynn, Norfolk. (1944.)
Gould, F. G., Woodrising, Trapps Hill, Loughton, Essex. (1918.)
Graddon, W. D., The Brooms, Park Lane, Congleton, Cheshire.
Grainger, Miss J., Wilshaw Road, Meltham, nr. Huddersfield. (1943.)
Grainger, J., B.Sc., Ph.D., Department of Plant Pathology, West of Scotland Agricultural College, Auchincruive by Ayr. (1944.)
Gray, Miss E. G., B.Sc., Ph.D., North of Scotland Agricultural College, 41½ Union Street, Aberdeen. (1942.)
Greenwood, E. L., Greenside, The Settlement, Ockbrook, Derby. (1944.)
Gregory, P. H., Ph.D., Rothamsted Experimental Station, Harpenden, Herts. (1930.)
Groves, J. Walton, Central Experiment Farm, Ottawa, Canada. (1942.)
Gwynne-Vaughan, Professor Dame Helen, G.B.E., D.Sc., LL.D., F.L.S., 93 Bedford Court Mansions, London, W.C. 1. (1906.)
Hanna, W. F., M.Sc., Ph.D., Dominion Rust Research Laboratory, Agricultural College, Winnipeg, Canada. (1925.)
Hansford, C. G., M.A., F.L.S., Mycologist, Department of Agriculture, Kampala, Uganda. (1921.)
Harley, J. L., M.A., D.Phil., Department of Botany, The University, Oxford. (1932.)
Harris, R.V., D.Sc., A.R.C.S., East Malling Research Station, nr. Maidstone, Kent. (1924.)

Harrison, T. H., D.Sc., Australia House, Strand, London, W.C. 2. (1931.)
Harvard University, The Library, Cambridge, Mass., U.S.A. (1923.)
Hastings, Somerville, M.S., F.R.C.S., 33 Murray Road, Northwood, Middlesex.
Hawker, Miss L. E., Ph.D., D.Sc., Botanical Department, The University, Bristol. (1934.)
Heim, Roger, Sous-Directeur au Muséum d'Histoire Naturelle, 11 Rue de Médicis, Paris (6e), France. (1930.)
Heimbeck, Mrs Louise, Brosoe, Levanger, Norway. (1923.)
Hickman, C. J., M.Sc., Botanical Department, The University, Edgbaston, Birmingham, 15. (1935.)
Hiscott, G. S., Furzy Grove, Kempshott Lane, Basingstoke, Hants. (1944.)
Hitchcock, Miss M. M., East Malling Research Station, nr. Maidstone, Kent. (1945.)
Holden, H. S., D.Sc., F.R.S.E., F.L.S., Forensic Science Laboratory, Shakespeare Street, Nottingham. (1923.)
Honolulu, Association of Hawaiian Pineapple Canners, P.O. Box 3166, Hawaii. (1929.)
Honolulu, The Library, Experimental Station, S.P.A., Box 411, Hawaii. (1920.)
Hora, Dr F. B., The University, Reading, Berks. (1943.)
Howard, H. J., F.R.M.S., F.L.S., Lingfield, 6 College Road, Norwich. (1918.)
Howe, F. J., F.R.M.S., Pathological Department, St Cross Hospital, Rugby, Warwickshire. (1943.)
Hubbard, Miss M. D., B.Sc., Laren, Western Drive, Littleover, Derby. (1933.)
Hughes, G. C., 20 Priory Road, Bicester. (1898.)
Hughes, J. S., M.A., The Firs, Great Rollright, Chipping Norton, Oxon. (1927.)
Hughes, S. J., B.Sc., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1941.)
Hull, R., B.Sc., Ph.D., A.R.C.S., Midland Agricultural College, Sutton Bonington, Loughborough. (1942.)
Hull, The Librarian, Botanical Department, University College. (1929.)
Humphrey, Dr C. J., 543 No. La Cienega Blvd., Los Angeles, Calif., U.S.A. (1921.)
Hurst, C. P., F.L.S., Landulph Rectory, Saltash, Cornwall. (1928.)
Ingold, Professor C. T., D.Sc., Ph.D., F.L.S., Department of Botany, Birkbeck College, Chancery Lane, London, E.C. 4. (1935.)
Iowa, The Library, State University of Iowa, Library Annex, Iowa City, U.S.A. (1923.)
Iowa State College, Library, Ames, Iowa, U.S.A. (1927.)
Isaac, I., B.Sc., East Malling Research Station, nr. Maidstone, Kent. (1943.)
Issatchenko, Professor Dr B. L., Nowinskii B. 25, log. 11, Moscow 69, U.S.S.R. (1923.)
Jenkins, Miss Anna E., Bureau of Plant Industry, Department of Agriculture, Washington, D.C., U.S.A. (1942.)
John Crerar Library, 86 East Randolph Street, Chicago, Illinois, U.S.A. (1929.)
Johnstone, R. H., 726 Anniesland Road, Glasgow, W. 4. (1938.)
Jones, G. H., M.A., Kasenga, Stanley Avenue, Chesham, Bucks. (1922.)
Jørstad, Ivar, Statsmykolog, Botanisk Museum, Oslo, Norway. (1923.)
Kapica, Mrs L., Rothamsted Experimental Station, Harpenden, Herts. (1943.)
Keay, Miss M. A., M.A., Ph.D., Norfolk Flax Establishment, Flitcham Abbey, Flitcham, King's Lynn, Norfolk. (1935.)
Keissler, Dr Karl, Direktor d. Botanischen Abteilung, Naturhistorisches Museum, Burgring 7, Wien 1/1, Austria. (1924.)
Keyworth, W. G., Ph.D., D.I.C., East Malling Research Station, nr. Maidstone, Kent. (1941.)
King, J. M. B., 23 Lyncroft Gardens, Ealing, London, W. 13. (1945.)
Klein, Richard, Ph.D., F.L.S., 3 Sussex Place, London, W. 2. (1943.)
Knight, Major Maxwell, O.B.E., The Homestead, Park Road, Camberley, Surrey. (1945.)
Knight, Mrs S., The Homestead, Park Road, Camberley, Surrey. (1945.)
Kuala Lumpur, F.M.S., The Director of Agriculture, Straits Settlements and Federated Malay States. (1930.)

Layton, T. A., 1 Keppel Street, London, W.C. 1. (1945.)
Leach, R., B.A., Department of Agriculture, Kingston, Jamaica. (1929.)
Legge, Miss B. J., Pinetrees, The Wergs, Wolverhampton. (1943.)
Leicester, The City Museum. (1923.)
Levisohn, Miss I., D.Phil., Forestry School, 6 Keble Road, Oxford. (1943.)
Linder, Dr D., Farlow Herbarium, Harvard University, 20 Divinity Avenue, Cambridge, Mass., U.S.A. (1935.)
Line, James, M.A., School of Agriculture, Cambridge. (1921.)
Linnean Society, The, Burlington House, Piccadilly, London, W. 1. (1919.)
Lloyd Library, The, 309 West Court Street, Cincinnati, 2, Ohio, U.S.A. (1907.)
Loader, Miss F. M., B.Sc., Botanical Department, University College, Southampton. (1927.)
Long, Mrs E. M. [née Ellis], B.A., B.Sc., 26 Constitution Hill, Norwich. (1930.)
Lumb, M., 18 Chaworth Road, West Bridgford, Nottingham. (1945.)
Lütjeharms, Professor W. J., Phil.Nat.D., Department of Botany, University College, Bloemfontein, Orange Free State, S. Africa. (1930.)
Macdonald, James A., Botany Department, The University, St. Andrews. (1938.)
McKay, Robert, B.Sc., A.R.C.S.I., Department of Plant Pathology, Albert Agricultural College, Glasnevin, Dublin. (1939.)
McQuillan, Miss C. M., Skipton Girls' High School, Skipton, Yorks. (1944.)
Marsh, R. W., M.A., Research Station, Long Ashton, Bristol. (1923.)
Masefield, G. B., c/o Department of Agriculture, Entebbe, Uganda. (1932.)
Mason, E. W., M.A., M.Sc., F.L.S., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1921.)
Mason, Mrs E. W., M.Sc., Inglenook, 63 King's Road, Richmond, Surrey. (1922.)
Mathias, W. T., B.Sc., The University, Liverpool. (1938.)
Matthews, Professor J. R., M.A., F.L.S., Department of Botany, The University, Old Aberdeen. (1921.)
Mehta, Professor K. C., Ph.D., Department of Biology, Agra College, Agra, U.P., India. (1921.)
Metcalfe, C. R., B.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey. (1926.)
Michigan Agricultural College Library, East Lansing, Michigan, U.S.A. (1924.)
Miller, Professor J. H., B.S., M.S., Ph.D., University of Georgia, Athens, Ga., U.S.A. (1930.)
Millidge, P. H., 205 Carisbrooke Road, Newport, I.O.W. (1937.)
Missouri Botanical Garden, The, St Louis, Mo., U.S.A. (1902.)
Montague, Mrs A., Penton, Crediton, N. Devon. (1898.)
Montgomery, H. B. S., B.A., Ph.D., D.I.C., East Malling Research Station, nr. Maidstone, Kent. (1940.)
Montreal, Institut Botanique de l'Université, 4101 Est, Rue Sherbrooke, Canada. (1932.)
Moore, Miss E. M., Wardrobes, nr. Lacey Green, Aylesbury, Bucks. (1943.)
Moore, Miss F. J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts. (1945.)
Moore, M. H., East Malling Research Station, nr. Maidstone, Kent. (1940.)
Moore, W. C., M.A., Ministry of Agriculture, Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (1922.)
Morris, L. E., c/o Eton College, Windsor, Berks. (1924.)
Moureau, J., Laboratoire de Phytopathologie, I.N.E.A.C., Bambesa, Belgian Congo. (1944.)
Muller, Dr H. R. A., c/o Internatio, Semarang, Java. (1932.)
Munro, Miss M. C. D., Ph.D., 12 Blantyre Terrace, Edinburgh, 10. (1945.)
Munson, R. G., 10 Abbotsbury Gardens, Eastcote, Pinner, Middlesex. (1943.)
Murray, G. H., F.E.S., Director of Agriculture, Rabaul, New Britain, Territory of New Guinea, via Australia. (1921.)

Muskett, Professor A. E., M.Sc., A.R.C.S., Queen's University, Belfast, Northern Ireland. (1923.)
Nannfeldt, Professor J. A., Sturegatan 11, Uppsala, Sweden. (1932.)
Nash-Wortham, J. R. H., Abingdon, Gatesden Road, Fetcham, Leatherhead, Surrey. (1937.)
National Collection of Type Cultures, Curator, Lister Institute, Elstree, Herts. (1921.)
National Museum of Wales, Cardiff. (1924.)
Nattrass, R. M., B.Sc. (Agric.), Ph.D., Department of Agriculture, P.O.B. 338, Nairobi, Kenya. (1925.)
Nederlandsche Mycologische Vereeniging, The Librarian, Zoornweg 10, Wageningen, Holland. (1920.)
Newcastle-on-Tyne, The Librarian, King's College. (1928.)
New York Botanical Garden, Bronx Park, New York, U.S.A. (1904.)
Nierenstein, M., D.Sc., 2 Rylestone Grove, Bristol, 9. (1945.)
Nobbs, Miss G. E. D., 4 Clifton Estate, Plympton, S. Devon. (1943.)
Noble, Miss Mary, B.Sc., Ph.D., 19*a* Willowbrae Avenue, Edinburgh. (1940.)
Noel, Miss E. F., F.L.S., 37 Burnham Court, Queen's Road, London, W. 2. (1913.)
North Carolina, Library, University of, Chapel Hill, North Carolina, U.S.A. (1920.)
Notley, Miss M. E., O.C.H.F., St Mary's Convent, Baldslow, St Leonards-on-Sea, Sussex. (1942.)
Nursery and Market Garden Industries' Development Society, Ltd., Experimental and Research Station, Cheshunt, Herts. (1922.)
O'Connor, P., Ph.D., B.Sc., A.R.C.Sc.I., National Museum, Dublin. (1925.)
Ogilvie, L., M.A., M.Sc., Research Station, Long Ashton, Bristol. (1922.)
Olliver, C. W., c/o Lloyds Bank Ltd., 50 Notting Hill Gate, London, W. 11. (1942.)
Ontario Agricultural College, Library, Guelph, Ontario, Canada. (1920.)
Osborn, Professor T. G. B., D.Sc., F.L.S., Department of Botany, The University, Oxford. (1910.)
Osborne, J. F., 21 Grimsdyke Road, Hatch End, Middlesex. (1944.)
Padwick, Dr G. Watts, Imperial Agricultural Research Institute, New Delhi, India. (1936.)
Page, Miss W. M., M.Sc., Ph.D., 5 Dartmouth Chambers, Theobald's Road, London, W.C. 1. (1921.)
Parker, Professor C. S., Department of Botany, Howard University, Washington, D.C., U.S.A. (1932.)
Paton, Miss M. R., Hareshawmuir by Kilmarnock, Ayrshire. (1945.)
Pearson, Arthur A., F.L.S., Nutcombe House, Hindhead Road, Hindhead, Surrey (1911.)
Peklo, Dr Jaroslav, Professor of Applied Botany, Bohemian Technical University, Charles Square, Prague II, Czechoslovakia. (1924.)
Pershouse, Mrs Stanley, Denhem Lodge, Yelverton, S. Devon. (1937.)
Perthshire Society of Natural Science, c/o J. F. Cumming, Esq., 12 Barossa Place, Perth. (1919.)
Pethybridge, G. H., O.B.E., Ph.D., B.Sc., F.L.S., Penlee, Harleigh Road, Bodmin. Cornwall. (1919.)
Pettifer, A. J., 36 Cheviot Gardens, Hendon Way, London, N.W. 2. (1944.)
Peyronel, Dr Benjamino, R. Istituto Sup. Agrario e Forestale, Piazzale del Re, Firenze, Italy. (1932.)
Philadelphia, The Academy of Natural Sciences of Philadelphia, Nineteenth and The Parkway, Phil., U.S.A. (1925.)
Phillips, Dr H. H., 11 Kings End, Bicester, Oxon. (1923.)
Pineapple Research Institute, The Library, P.O. Box 3166, Honolulu, Hawaii. (1920.)
Ping, A. Wentworth, M.A., St Olave's, Clifton, York. (1926.)
Plunkett, B. E., B.Sc., Botany Department, The University, Old Aberdeen. (1945.)

Potter, Rev. M. C., Sc.D., M.A., F.L.S., Corley Croft, New Milton, Hants. (1896.)
Prentice, I. W., B.Sc., East Malling Research Station, nr. Maidstone, Kent. (1943.)
Preston, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop. (1920.)
Pretoria, South Africa, The Librarian, Division of Botany and Plant Pathology, P.O. Box 994. (1922.)
Ramsbottom, J., O.B.E., Dr.Sc., M.A., F.L.S., British Museum (Nat. Hist.), Cromwell Road, South Kensington, London, S.W. 7. (1910.)
Rauter, Dr F., 28 Clarendon Road, London, W. 11. (1942.)
Ray, Miss Anne, Penarwyn, Gorran Haven, Gorran, Cornwall. (1929.)
Rayner, Dr M. Cheveley (Mrs Neilson Jones), Bedford College for Women, Regent's Park, London, N.W. 1. (1921.)
Rees, John, M.Sc., Adviser in Agricultural Botany, University College, Cardiff. (1929.)
Reichert, Dr Israel, Jewish Agency for Palestine, Agricultural Experiment Station, P.O.B., 15 Rehoboth, Palestine. (1924.)
Rhodes, Miss Mabel, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
Robertson, N. F., 61 East Trinity Road, Edinburgh, 5. (1945.)
Robinson, E., 26 Burwood Avenue, Eastcote, Pinner, Middlesex. (1938.)
Rogers, Dr D. P., Department of Biology, American International College, Springfield, Mass., U.S.A. (1943.)
Rose, H. V., Morden College, Blackheath, London, S.E. 3. (1944.)
Rothamsted Experimental Station, Department of Plant Pathology, Harpenden, Herts. (1923.)
Rothwell, M. S., Africa House, Kingsway, London, W.C. 2. (1945.)
de Rousset-Hall, O., B.A., Low Temperature Research Station, Cambridge. (1942.)
Royal Holloway College, Englefield Green, Surrey. (1942.)
Rutgers College and State University of New Jersey, Library, New Brunswick, New Jersey, U.S.A. (1922.)
St Paul, Minnesota, U.S.A., The Library, Department of Agriculture, University Farm. (1920.)
Sampson, Miss K., M.Sc., Malmsmead, Lacey Green, nr. Aylesbury, Bucks. (1920.)
Samuel, Geoffrey, M.Sc., Ministry of Agriculture, Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (1923.)
Schimmer, Miss F. C., M.Sc., Wye College, Ashford, Kent. (1943.)
Scott, W. W., 13 Bishop's Road, Highgate, London, N. 6. (1922.)
Searle, G. Odell, B.Sc. (Agric.), Flax Research Institute, Flitcham Abbey, Flitcham, King's Lynn, Norfolk. (1920.)
Seth, N. L., B.Sc., Ph.D., D.I.C., Agricultural College, Mandalay, Burma. (1930.)
Shear, Dr C. L., U.S. Department of Agriculture, Bureau of Plant Industry, Washington, D.C., U.S.A. (1930.)
Sheard, Mrs E., Experimental and Research Station, Cheshunt, Herts. (1937.)
Skene, Professor Macgregor, Department of Botany, The University, Bristol. (1936.)
Smith, Alexander, M.A., Ph.D., Ministry of Agriculture, Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (1924.)
Smith, G., M.Sc., F.R.I.C., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1936.)
Smith, Miss K. Goldie, 12 Victoria Park Road, Leicester. (1944.)
Smith, Professor Noel J. G., Ph.D., B.Sc., Botany Department, Rhodes University College, Grahamstown, S. Africa. (1924.)
South London Botanical Institute, 323 Norwood Road, Tulse Hill, London, S.E. 24. (1921.)
Sowter, F. A., F.L.S., 9 North Avenue, Leicester. (1942.)
Stakman, Professor E. C., University of Minnesota, Department of Agriculture, University Farm, St Paul, Minn., U.S.A. (1922.)

Stationery Office, H.M., Superintendent of Publications, Book Dept., Westminster, S.W. 1. (5 subscriptions.) (1920.)
Stephens, Miss E. L., B.A., Department of Botany, University of Cape Town, South Africa. (1928.)
Sterry, Miss M., B.Sc., Manor Cottage, Longhope, Glos. (1942.)
Stevenson, Dr J. A., U.S. Department of Agriculture, Bureau of Plant Industry, Washington, D.C., U.S.A. (1927.)
Stewart, Miss S. B., Insects Pests Laboratory, 30 High Street, Steyning, Sussex. (1944.)
Steyaert, R. L., Ing. A.I.Gx., Laboratoire Bambesa, Uele, Belgian Congo. (1931.)
Stiles, Professor W., Sc.D., F.R.S., Botanical Department, The University, Edgbaston, Birmingham, 15. (1936.)
Stirrup, H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough. (1922.)
Storey, H. H., M.A., Ph.D., East African Agricultural Research Institute, Amani, Tanganyika Territory, East Africa. (1922.)
Storey, I. F., Ph.D., Department of Agriculture, The University, Leeds, 2. (1941.)
Swanton, E. W., M.B.E., A.L.S., Educational Museum, Haslemere, Surrey. (1899.)
Swedish Academy of Sciences, Royal, Stockholm, Sweden. (1919.)
Sydney, Australia, The Librarian, University of. (1922.)
Tennessee, University of, Agricultural Experiment Station, Library, Knoxville, Tennessee, U.S.A. (1926.)
Tervet, I. W., B.Sc., Department of Plant Pathology, University Farm, St Paul, Minn., U.S.A. (1933.)
Thirumalachar, M. J., M.Sc., D.Sc., 20th V Main Road, Malleswaram, Bangalore, India. (1945.)
Thomas-Perrott, Mrs P. E., Department of Agricultural Botany, University College of Wales, Aberystwyth. (1945.)
Thompson, Miss E. C., East Malling Research Station, nr. Maidstone, Kent. (1942.)
Thompson, Miss M. V., Pathological Research Laboratory, Christie Hospital and Holt Radium Institute, Manchester, 20. (1942.)
Thurston, Miss J. M., B.Sc., Rothamsted Experimental Station, Harpenden, Herts. (1942.)
Tipler, R. V., B.Sc., A.R.C.S., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1945.)
Tomkins, R. G., M.A., Ph.D., Trinity College, Cambridge. (1925.)
Tunstall, A. C., Tocklai Experimental Station, Cinnamara P.O., Assam, British India. (1933.)
Turner, Miss E. M., B.A., Ph.D., Old School House, Great Haseley, Oxon. (1940.)
Turner, Mrs F. M., Rosecot, 26 Halls Road, Tilehurst, Reading, Berks. (1943.)
Twyman, E. S., Botanical Department, The University, Edgbaston, Birmingham, 15. (1942.)
Vanterpool, T. C., M.Sc., Botanical Department, University of Saskatchewan, Saskatoon, Canada. (1929.)
Venkatarayan, S. V., Mycologist, Agricultural Department, Bangalore, S. India. (1935.)
Wadham, Professor S. M., M.A., Department of Agriculture, The University, Melbourne, Victoria, Australia. (1922.)
Waldie, J. S. L., B.Sc., C.D.A., Department of Agricultural Botany, The University, Reading. (1925.)
Wales, University College of, Librarian, Botanical Department, Aberystwyth, North Wales. (1927.)
Wallace, E. R., Agricultural Institute, Kirton, nr. Boston, Lincs. (1934.)
Wallace, G. B., B.Sc. (Agric.), Ph.D., Lyamungu, Moshi, Tanganyika Territory, E. Africa. (1928.)
Wardlaw, Professor C. W., Department of Cryptogamic Botany, The University, Manchester, 13. (1943.)

Ware, W. M., D.Sc., Wye College, Ashford, Kent. (1924.)
Warne, Mrs M. M., B.A., 25 Amherst Road, Fallowfield, Manchester, 14. (1937.)
Washington, State College of, Pullman, Wash., U.S.A. (1924.)
Waterhouse, Miss G. M., M.Sc., 95 Knightwood Crescent, New Malden, Surrey. (1927.)
Waterston, J. M., B.Sc., Mycologist, Department of Agriculture, Paget East, Bermuda. (1934.)
Watson, W., D.Sc., A.L.S., Cedene, Cheddon Road, Taunton. (1923.)
Webb, Dr Phillip, The Yews, Chadlington, Oxford. (1938.)
Webb, R. A., B.A., D.Phil., National Hospital, Queen's Square, London, W.C. 1.
Westerdijk, Professor Johanna, Javalaan 4, Baarn, Holland. (1923.)
Western, J. H., B.Sc., Ph.D., Adviser in Mycology and Agricultural Botany, The University, Manchester, 13. (1934.)
Weston, W. A. R. Dillon, M.A., Ph.D., School of Agriculture, Cambridge. (1923.)
Whitaker, F. Owen, 51 Grosvenor Avenue, Carshalton, Surrey. (1921.)
White, Neville, Tasmanian Department of Agriculture, Box 673E, G.P.O., Hobart, Tasmania. (1944.)
Whitehead, T., D.Sc., A.R.C.S., University College of North Wales, Bangor. (1920.)
Wilkins, W. H., M.A., D.Phil., Department of Botany, The University, Oxford. (1928.)
Wilkinson, E. H., Research Station, Long Ashton, Bristol. (1938.)
Williams, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts. (1930.)
Wilson, Miss A. P., M.B.E., A.R.C.S., 7*a* Arlington Drive, Nottingham. (1929.)
Wilson, Alastair R., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough. (1933.)
Wilson, Miss Irene M., B.Sc., Ph.D., Botany Department, University College of Wales, Aberystwyth. (1938.)
Wilson, Malcolm, D.Sc., A.R.C.S., F.L.S., Royal Botanic Garden, Edinburgh. (1912.)
Wiltshire, S. P., D.Sc., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1920.)
Wisconsin, The Library, University of, Madison, Wis., U.S.A. (1923.)
Wolf, Dr F. A., Department of Botany, Duke University, Durham, North Carolina, U.S.A. (1940.)
Wolf, Dr F. T., Vanderbilt University, Nashville, Tennessee, U.S.A. (1940.)
Wood, F. C., The Rest, Franklin Road, Durrington, Worthing. (1935.)
Woodcock, A. J. A., M.Sc., F.E.S., Rhianva, 65 Rock Avenue, Gillingham, Kent. (1926.)
Woodward, R. C., Ph.D., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1924.)
Woolhope, The Naturalists' Field Club, Hereford. (1896.)
Wormald, H., D.Sc., A.R.S.C., East Malling Research Station, nr. Maidstone, Kent. (1921.)
Yale University, Library, New Haven, Connecticut, U.S.A. (1930.)
Yuill, E., Norwood, Towthorpe Lane, Haxby, York. (1937.)
Zürich, Switzerland, Botanical Garden and Museum, c/o Dr A. U. Däniker. (1921.)
Zürich, Institut für Spezielle Botanik der Eidg. Techn. Hochschule. (1928.)

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